STUDIES ON RESISTANCE TO *FASCIOLA HEPATICA* IN RATS AND RABBITS

by

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To the memory of

ELGASIM MOHAMED HAROUN

(1947 - 1975)

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PREFACE

The work described in this thesis is original and has not been submitted in any form to any other University. It was carried out by the author in the Department of Tropical Animal Health, Royal (Dick) School of Veterinary Studies, University of Edinburgh, under the supervision of Dr. M.M.H. Sewell and Dr. J.A. Hammond.

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SUMMARY

Following a review of the literature on resistance to Fasciola hepatica, a series of experiments are described, which were designed to study the protective effect of curtailed previous infections, implantation with adult flukes and passive transfer of immune serum in rats and rabbits. Rats were chosen to represent those mammals which readily acquire resistance to F. hepatica e.g. cattle, and rabbits to represent those in which the ability to acquire such resistance is questionable e.g. sheep.

One mature or two immature infections which had been eliminated with deacetylated diamphenethide stimulated similar levels of resistance to challenge with F. hepatica in rats, although the latter procedure resulted in more severe hepatic lesions than the former. In both cases the resistance was manifested by a significant decrease in the numbers of flukes recovered from previously infected rats compared with previously uninfected rats. Following challenge infections, the resistant rats also showed significantly lower mean peak eosinophil counts and significantly lower mean peak glutamic dehydrogenase levels, indicating less liver damage. Higher antibody titres were also recorded after challenge in the previously infected animals than in the controls.

On the other hand, in rabbits neither a single mature or two previous immature infections which had been eliminated with rafoxanide stimulated a significant resistance to challenge. The two previous infections appeared to result in some decrease in the challenge infection but this was not

statistically significant and was attributed to structural changes in the liver acting as a mechanical barrier to the development of the challenge infection. Although such structural changes resulting from previous infections may also play a role in resistance to F. hepatica in rats, the involvement of immunological factors in this resistance was clearly demonstrated by implanting adult flukes subcutaneously or intraperitoneally thus by-passing the parenchymal intrahepatic migration which is the probable stimulus resulting in the mechanical barrier. These implantations resulted in a significant resistance to challenge in rats but not in rabbits. Once again, the resistance was manifested by both a significant reduction of the challenge infection and a significant decrease in the mean peak serum glutamic dehydrogenase levels after challenge.

In rats metabolic products diffusing from mature flukes which had been encapsulated in diffusion chambers and implanted subcutaneously or intraperitoneally were found to contain immunogens which stimulated resistance to challenge. Hence, the fluke tegumental cells or the eggs do not appear to have an essential role in this resistance. The similar stimulation of resistance by subcutaneous implantation of one or two encapsulated adult flukes, which were removed after two weeks, showed that the continuing presence of the sensitising flukes was not needed to maintain the resistance, at least in the short term.

It was also shown that resistance can be transferred passively by the globulin fraction of serum and therefore

probably by antibody. This protective agent in immune serum or immune-gamma globulin could be inactivated or absorbed out by factor(s) present in the metabolic products of cultured flukes.

The successful transfer of resistance by immune serum seems to depend on the volume of serum transferred, the time of transfer in relation to infection and the species of the recepient and the donor. Thus immune rat serum or gamma-globulin precipitated from such serum, transferred at the time of challenge and again 2 days later, resulted in a significant level of resistance in terms of both the number of flukes recovered from the challenge infection and the serum glutamic dehydrogenase levels. However such resistance was not obtained in rabbits injected with immune homologous serum. The ratio of the transferred serum to recepient's weight was about 1:50 in rabbits compared to 1:15 in rats. However, using these same ratios immune rat serum was found to confer protection on rabbits and immune rabbit serum was protective to rats. This suggests that the immune rat serum simply has a greater protective capacity. A similar high protective capacity was also shown by immune bovine serum and by gamma-globulin precipitated from such serum but not by ovine serum.

The dissertation ends with a discussion of these results and of their relevance to the various hypotheses which have been advanced to explain the mechanism of resistance to Fasciola hepatica.

CHAPTER ONE

INTRODUCTION

Fascioliasis is the disease caused by digenetic trematodes of the genus Fasciola. In domestic livestock there are two generally recognised species but there are others of disputed validity (Dinnik and Dinnik, 1959; Sinclair, 1967; Hammond 1970). The type species F. hepatica (Linnaeus, 1758) is of very wide distribution mainly in the temperate parts of the world, Australia and the Americas but also at high altitudes (over 1670 met) in tropical areas (Dinnik and Dinnik, 1959; Kendall, 1965).

The life cycle of F. hepatica was first described by Leuckart (1882) and Thomas (1883). Sinitsin (1914) elucidated the route of migration from the intestine to the liver and Schumacher (1938) confirmed that F. hepatica migrates across the peritoneal cavity into the liver. The second major species is F. gigantica (Cobbold, 1855), which is widespread in the tropics and sub-tropics.

Fascioliasis is economically most important in sheep and cattle, but it occurs in a very wide range of susceptible mammals including goats, camels, buffalo, swine and equidae. Wild mammals may also contract the disease and some of these act as reservoir hosts (Dinnik and Dinnik, 1959; Hammond, 1972). There are also reports of human fascioliasis from many parts of the world (Dawes and Hughes, 1964; Boray, 1969; Hammond, 1974).

Dargie (1975) summarised the direct effects of the disease as a) antigenic stimulation, leading to elevated serum

globulins and eosinophilia b) liver damage, resulting in elevated serum enzymes, anaemia and hypoalbuminaemia c) inappetance, with a consequent loss of weight or poor weight gain d) Haemorrhage via the bile due to the blood sucking activities of adult parasites. The importance of these effects are mainly influenced by the number of flukes in the host.

Sewell (1966a) pointed out that F. gigantica appears to be better adapted as a parasite of cattle than F. hepatica and that the fibrosis and calcification caused in the livers of cattle infected with the former is less marked than in those infected with the latter, so that the livers appear more like those of sheep infected with F. hepatica. However, this author also commented that otherwise there is no great difference between the two species in the aetiology of either the subacute form of the disease associated with the parenchymal migration of large numbers of immature flukes or the more chronic form due to the adult flukes within the bile ducts. However, the infectivity of both species and the development of the disease are influenced by various factors such as the breed, age, nutritional status and management of the host as well as the ecological requirements of the different intermediate hosts, these being the small amphibious Lymnaea truncatula (Müller) for F. hepatica or the larger aquatic snail L. natalensis (Krauss) for F. gigantica (Sewell and Hammond, 1974).

The economic losses due to fascioliasis may be very serious, particularly where the local environmental conditions are

favourable to the intermediate hosts, so that the final hosts are exposed to a high rate of infection which may lead to outbreaks of acute fascioliasis with both high morbidity and high mortality (Sewell, 1974). However, of greater overall economic importance is the chronic wasting condition. In sheep the sequelae include poor fleece yields (Roseby, 1970), as well as low birth weights, low milk production by the ewe and subsequently poor weight gains by her lambs (Reid, 1973).

Chronic infections of cattle result in poor food conversion ratios (Liddel, 1972) lowered weight gains and poor milk production in terms of both quantity and quality (Ross, 1970; Black and Froyd, 1972). In addition, condemnation of infected bovine and ovine livers at meat inspection results in a significant loss of revenue.

In the present author's experience, fascioliasis is the most important helminth disease in the Sudan and undoubtedly accounts for serious economic losses as a result of mortality, reduced productivity and the condemnation of infected livers (Karib, 1962; Haroun and Hussein, 1975). The predominant parasite is F. gigantica but it is not known whether F. hepatica is present at higher altitudes towards the frontier with Ethiopia and Jebel Marra area in the western Sudan. The disease is endemic all along the areas irrigated by the White Nile and its tributaries from the southern borders of the country to Khartoum.

It is also reported from the western parts of the country where permanent swamps and shallow water-bodies exist.

The situation is aggravated by the scarcity of grazing land during the dry season, particularly in the western provinces, which have the largest livestock population in the country. The result is that large populations of animals are concentrated along the White Nile and its tributary Bahr El Ghazal where they are exposed to infection (Haroun, 1975).

Significant advances have been made in the control of fascioliasis by the development of more effective fasciolicides and molluscicides as well as by improvement of land drainage and disease forecasting techniques in the more developed parts of the world. However, control methods need repeated treatments and are therefore expensive and in general have limited application in countries such as the Sudan. Hence, the development of an effective vaccine against this disease would be of great value.

The objectives of the present investigation were therefore to extend knowledge of means of immunising experimental animals against F. hepatica either actively or passively and to monitor the effects of this resistance using appropriate parameters. Secondly, to further study the role of soluble metabolic products in the stimulation of resistance against F. hepatica.

Rats and rabbits were used as models for cattle and sheep in this work as they offer both logistic and financial advantages, although it is appreciated that there will be a need to confirm observations made in laboratory hosts in the definitive host of commercial importance.

CHAPTER 2

REVIEW OF THE LITERATURE ON RESISTANCE TO FASCIOLA HEPATICA

It has been established that many mammalian hosts naturally possess or can acquire an ability to resist a wide range of helminthic infections (Soulsby, 1958; Urquhart, Jarrett and Mulligan, 1962). Immunity is conveniently divided into two forms: innate immunity and acquired immunity. The former is an expression of the host's ability to resist infection without previous contact with the infective agent while the latter requires such contact. Both types of immunity are involved in suppressing fascioliasis and may be expressed in a number of ways e.g. reduction in the number of flukes reaching the liver parenchyma or bile ducts, retardation or inhibition of development of flukes in the liver, elimination of the parasites in the parenchyma during the immature phase or reduction of the life span of the adult parasites in the bile ducts (Armour and Dargie, 1973; Ross, 1967a).

2.1. Innate resistance

Almost all mammals are susceptible to Fasciola hepatica (Kendall, 1967). This susceptibility, however, varies considerably from species to species depending on a number of factors including: the histological structure of the host's liver, the type and severity of the reaction produced by the parasite in the liver and the level of infection (Ross, 1967a, Boray 1967a, Nansen, Anderson, Harmer and Riising, 1972).

Ross (1967a) classified mammalian hosts into three groups

on the basis of their innate resistance to F. hepatica: low, medium and high resistance groups. Laboratory animals, sheep and possibly horses and donkeys are included in the first group, cattle are considered to have medium resistance whereas pigs are highly resistant.

2.1.1. Rats

Innate resistance to Fasciola hepatica in rats is dependent on various factors which include age, sex, strain of rat and the size of the infective dose.

2.1.1.1. Age

Boray (1964) reported that whereas infections with F. hepatica developed in 90-93% of young Wistar strain rats, no adult flukes were recovered following infection of 5 to 6 month old rats with 5-10 metacercariae. Ray (1970) also noted that male rats weighing 65 to 75 g were more susceptible to infection with F. hepatica than older animals weighing 160 to 170 g. Similarly, Armour and Dargie (1973) and Dargie (1973) reported that adult rats are more resistant to fascioliasis than young animals. They observed that age resistance was coincident with the formation of hepatic fibrous tissue, but they did not discount the possibility of immunological immaturity acting as a contributory factor in the greater susceptibility of young animals.

Hayes, Bailer and Mitrovic (1974a) also examined the effect of age on resistance to F. hepatica in male rats. Eight and 13-month-old rats exposed to 20 cysts were found to yield 58 and 56% less flukes, respectively, than similarly infected 3 or 4-week-old rats. This difference was significant.

Goose and Macgregor (1974) observed that age resistance only developed after puberty. They found that susceptibility to infection with F. hepatica in Wistar rats increased with age from weaning to a peak at puberty. The time at which age resistance manifests itself was also investigated by Rajasekariah and Howell (1977a). They encountered a variation in the degree of resistance in 10-week-old rats, which led them to suggest that the factors involved in age resistance in rats develop at about that age. These authors also observed that age resistance was consistently demonstrated when worm recoveries from 15 and 25-week-old rats were compared with those from 5-week-old animals. They also found no difference in worm recoveries from 15-week and 25-week-old rats and concluded that once age resistance is fully established it does not become further enhanced.

Age resistance in the rat may also result in elimination of the fluke burden. Thus Boray (1964) found that his rats lost their fluke burden 4 months after infection. However, Hayes et al. (1974a)reported that some outbred Charles River strain albino rats only lost their fluke burden 12 months after infection, while Hughes, Harness and Doy (1976) also reported that inbred Piebald Virol Glaxo and Sprague Dawley strains eliminated their fluke burden 7 and 18 months respectively after challenge.

2.1.1.2. Sex

Ray (1970) reported that the sex of the rat did not appear to affect their susceptibility to infection with F. hepatica. However, female rats were reported to be more resistant to

F. hepatica than males by Dargie (1973) and Armour and Dargie (1973).

Later Goose and Macgregor (1974) reported that whereas males are more susceptible to infection than females at puberty they found no difference in susceptibility in weanling or mature Wistar strain rats. Furthermore, according to Hughes, Harness and Doy (1976), whereas sex difference affected the susceptibility of Piebald Virol Glaxo strain to infection with F. hepatica, it did not in the Sprague Dawley strain.

2.1.1.3. Strain

According to Wakelin (1970) the strain of the host can greatly influence the course of experimental infections with a particular species of parasite. This effect was reported with the nematode Trichuris muris to range from the inability of the parasite to achieve patency in some strains of mice to normal development in others. A strain difference was also reported in the response of rats to F. hepatica infection by Hughes, Harness and Doy (1976), as three or four times as many flukes develop in Piebald Virol Glaxo rats than in Sprague Dawley rats. Furthermore, Piebald Virol Glaxo rats eliminated their fluke burden 7 to 8 months after infection as compared with the 18 months needed by the Sprague Dawley rats. These authors considered that this difference might be a function of the greater number of flukes which reach the bile ducts in the former strain. Hence, they postulated the existence of a threshold number of flukes for each strain which is necessary for the stimulation of elimination.

2.1.1.4. Dose of Metacercariae

Thorpe (1965) reported that in rats infected with a large number of metacercariae of F. hepatica there was some delay in the flukes entering the bile ducts. He injected five groups of male Wistar rats with 5, 20, 40, 80 and 160 metacercariae respectively. Six weeks after infection he found that only in the group injected with 5 metacercariae was a greater proportion of flukes recovered from the bile ducts than from the liver parenchyma although a small number of flukes was recovered from the bile ducts even in the group infected with 160 metacercariae. This led him to the conclusion that some form of crowding effect or competitive inhibition by flukes takes place in heavy infections. Conversely when Rajasekariah and Howell (1977a) injected groups of male Wistar rats aged either 5, 10, 15 or 25 weeks with 1, 5, 10, 15 or 20 metacercariae they found that the proportion of worms that developed to maturity at a particular age was the same in all groups. Moreover, they found consistency in prepatent periods and overlap in worm sizes from various groups. They therefore concluded that, over their narrow range of rather low doses, neither a crowding effect nor competitive inhibition occurred and suggested that these phenomena may only occur in rats infected with large numbers of metacercariae.

2.1.2. Rabbits

The use of rabbits in experimental infections with F. hepatica was first reported by Lutz (1892). Much later Urquhart (1954) found that the susceptibility of rabbits to F. hepatica varies within a wide range. Thus the number of

flukes recovered from 32 rabbits infected with a total of 1600 metacercariae of F. hepatica ranged from 8% to 70% of the infecting dose with a mean of 32.2%. These findings were comparable to those of Montgomerie (1928) in sheep. When this author infected 32 sheep with a total of 2550 metacercariae, he observed a mean fluke burden of 37.4% of the infecting dose with a range between 8% and 76%. Hence, Urquhart (1954) suggested that the innate resistance of sheep to infection with F. hepatica is at about the same level and within the same limits as that of rabbits. He maintained that sex and breed have no significant effect on the susceptibility of rabbits to F. hepatica, although he noted that this comparison had been made between females and castrated males and also that the numbers of rabbits of the two breeds used (Dutch and Old English-Chinchilla crosses) were not ideally distributed to determine their precise importance.

The lack of any age resistance in rabbits was also pointed out by Kendall, Hebert, Parfitt and Peirce (1967). As they recovered a lower proportion of flukes from larger doses of cysts, this suggested that this might be a manifestation of competitive inhibition similar to that reported in rats (Thorpe, 1965).

2.1.3. Mice

The susceptibility of mice to F. hepatica was first reported by Shirai (1927) and Taylor and Parfitt (1957) found this host suitable for viability tests of metacercariae. Infections with 10 to 20 metacercariae were reported by Hughes (1962a) to be fatal within 3 to 4 weeks. This is caused by severe damage to the hepatic parenchyma which is greatest

around the 5th week after infection when the parasite exhibits maximum growth (Dawes and Hughes, 1964). Later, Harness, Doy and Hughes (1977a) showed that there is no advantage to be gained by infecting mice with more than 2 metacercariae if adult infections are required. They infected groups of mice orally with 2, 3 or 5 metacercariae. Eleven weeks later, they found the mortality rates to be 40%, 62% and 77% respectively.

The strain of mouse was shown by Harness, Doy and Hughes (1973) to affect susceptibility to infection with F. hepatica. When these authors infected bacteria susceptible, virus susceptible (BSVS) albino mice and Laboratory Animals Centre (LAC) grey mice with 20 metacercariae each and examined them at 24-hour intervals after infection, they found a quicker migration of flukes across the body cavity and earlier penetration of the liver in the LAC mice than in the BSVS strain.

2.1.4. Sheep

There is no evidence of natural resistance to F. hepatica in sheep (Taylor, 1949). According to Durbin (1952) infection can persist for at least 11 years compared to 3 years in rabbits (Montgomerie, 1931) and 4 years and 9 months in goats (Leiper, 1938). Ross (1967a) observed little or no obstruction to the migration of the immature parasites in the ovine hepatic parenchyma. Fibrosis was minimal and liver cell regeneration was found to be characteristic of the ovine liver. Fibrosis was also found to be minimal after the establishment of the flukes in the bile ducts, which expand to accommodate

increasing numbers of parasites.

As with mice (Dawes and Hughes, 1964), high level infections have been reported to be fatal in the critical phase between the 6th and 8th week during which maximum damage to the liver occurs (Ross, 1967a).

2.1.5. Cattle

The relatively high natural resistance of cattle to F. hepatica has been recognized for a long time (Hutyra and Marek, 1926; Taylor, 1949). This resistance is expressed both during the migratory parenchymal and the adult bile duct stages of the infection (Ross, 1965 a, b; 1967a; Boray, 1967a). According to Ross (1967a), the former is related to the relatively large amount of fibroelastic tissue in the bovine liver, which helps to restrict and inhibit the parasite's migration in the parenchyma. The bile duct resistance, characteristic of cattle, is thought to be due to the marked fibrosis and calcification of the bile ducts resulting in starvation of the flukes and their eventual elimination 16-30 weeks after infection. This expulsion is preceded by a period of 4 to 6 weeks of reduced biological activity, resulting in diminished faecal egg output (Doyle, 1971).

Ross (1965a) investigated the effect of the size of the infective dose and reported that a dose of 2500 cysts or more results in a drastic reduction in the number of flukes reaching the bile ducts and that at even higher doses of 5000 - 15000 yet more immature flukes are trapped in the liver parenchyma. Hence, as the level of infection is increased, this retardation and inhibition reduces the numbers of adult

flukes which develop. The author suggested that this is caused by a high degree of tissue reaction and intensive fibrosis, which is specially marked in the ventral lobe of the liver due to preferential migration by the parasites.

According to Ross (1968) the life span of F. hepatica in cattle is a function of the level and pattern of infection becoming shorter with increasing levels of infection. Thus, whereas from a single infection of about 200 cysts, 60 adult parasites were established and 75% of these were lost between the 5th and 21st month after infection and the rest survived up to the 26th month; with a single infection of 2500 to 15000 cysts, however, the life span of adult parasites in the bile ducts was reduced to 6-7 months (Ross, 1967b).

Ross (1968) also observed that 14-17-month-old steers were more resistant to initial heavy infections than younger animals and that calves in particularly good condition showed more resistance than those in poor condition.

2.2. Acquired resistance

Several ways of stimulating resistance to F. hepatica have been tried in various hosts. These include repeated infections with normal metacercariae, infection with irradiated metacercariae, inoculation with dead fluke material (somatic antigens), inoculation with secretions and excretions of living flukes (metabolic antigens), implantation with living flukes, passive transfer of immune serum, passive transfer of lymphoid cells and exposure to other (less pathogenic) parasites.

2.2.1. Stimulation of resistance by repeated infections with F. hepatica

2.2.1.1. Rats

There is now substantial experimental evidence indicating that rats are able to develop a singificant degree of resistance to reinfection with F. hepatica. As in innate resistance, acquired resistance has been found to be stronger in the female than in the male host (Goose and Macgregor, 1974).

Ruther (1963) demonstrated that 5 immunising infections each with 50 metacercariae caused a significant reduction in mean fluke burden to 0.75% of the infecting dose compared to 17.6% in rats with a single infection. The size of flukes from resistant rats was also reduced to about 50% of those from control infection. Immunising and challenge infections were all performed after leaving time for regeneration of liver tissue.

Hayes, Bailer and Mitrovic (1972) reported a reduction of 92.5% in the parasitic burden of rats previously infected with 5 metacercariae each of F. hepatica and challenged with a similar dose after 7 weeks, when compared with controls. They distinguished the parasites of the immunising and challenge infections by size and found no detectable effect on the primary infection by the challenge infection.

Hayes, Bailer and Mitrovic (1973) confirmed their 1972 findings by reporting a reduction of 76% in the number of flukes recovered from challenge infections of 10 metacercariae

after a primary infection with only one fluke. Rats with primary infection of 10 metacercariae were reported to have completely resisted superinfection. Challenge infections were again found not to affect the primary infections in terms of parasitic burden, in spite of the profound effect of the primary infection on challenge.

Further evidence of resistance to superinfection as well as the presence of this resistance in long standing chronic infections was reported by Hayes, Bailer and Mitrovic (1974a). They carried out a study in which rats were challenged with 20 metacercariae of F. hepatica 28 or 48 weeks after initial exposure to the same dose. Significant differences in the parasitic burdens recovered from superinfected rats and controls were found in both occasions.

Hayes et al. (1972; 1973; 1974a) recovered these significantly reduced fluke burdens from previously infected rats 3 or 4 weeks after challenge and concluded that the expression of resistance must have had taken place earlier than that time. According to Hayes et al. (1972) this resistance was not a function of the host's age as there was no difference in the proportion of an initial infective dose which developed in rats infected at 3.5 or 10.5 weeks of age. To further determine the time of expression of this resistance, Hayes and Mitrovic (1977) carried out a study in which they exposed rats to primary infection with 15 metacercariae of F. hepatica and superinfected them with 300 metacercariae 7 to 11 weeks later. When challenge infections were

terminated after 24 or 48 hours, 83.9% to 99% fewer flukes were recovered from the peritoneal cavities of superinfected rats than from rats not previously exposed. Hence, they reported that resistance to F. hepatica in the rat is expressed within the first 24 hours after challenge. Doy, Hughes and Harness (1978) also reported that a 3-week-old primary infection of 30 metacercariae per rat initiated a high degree of resistance within 48 hours after challenge with 200 cysts. A mean of 0.8 flukes was recovered from the peritoneal cavities of the previously infected rats as compared to 39.2 flukes from control rats.

Removal of the immunising infection by anthelmintic treatment before challenge did not appear to interfere with the ability of rats to develop resistance against challenge. This was demonstrated by Goose and Macgregor (1973a). Rats infected with 30 metacercariae of F. hepatica each and re-infected with the same dose after 9 or 14 weeks harboured significantly fewer flukes from the challenge infection than the previously uninfected controls. Autopsy was carried out 3 weeks after challenge. A similar level of resistance was found whether or not the rats were treated with rafoxanide to remove the initial infection 4 and 6 days before challenge. This resistance was not only directed against immature flukes derived from oral infection but also against adult flukes transferred into the peritoneal cavities of immunised rats. Thus rats implanted intraperitoneally with 5 or 6 adult flukes 9 or 10 weeks after an immunising oral infection of 30 cysts of F. hepatica harboured significantly fewer implanted flukes

than controls when they were killed a week after implantation. Comparable results were obtained by Hughes, Anderson and Harness (1976). They implanted 3 flukes in the peritoneal cavities of each of 16 rats which had been infected orally with 30 metacercariae of F. hepatica 10 weeks previously. Of these 34 were found to be dead at necropsy one week after implantation, whereas only 3 of the 48 flukes implanted in previously uninfected controls were dead. In contrast, when 10 flukes were implanted subcutaneously into another 10 rats, which had been orally infected with 30 metacercariae 10 weeks previously, 9 were found alive at necropsy 7 weeks later, although they were all encysted and degenerative changes were found in their testes. These results led Hughes, Anderson and Harness (1976) to the conclusion that oral infection causes sensitization of the host with all stages of the flukes, that this is necessary for the development of protection against adult fluke challenge and that this protection only appears to express itself fully when the challenge flukes are placed in the body cavity. According to Hughes, Harness and Doy (1977a; b) the resistance starts 2 weeks after sensitization, is affected by the duration of the sensitizing infection but is independent of the number of flukes present in the bile ducts. They reported loss of the ability to kill intraperitoneally implanted adult F. hepatica or F. gigantica in rats sensitised orally by F. hepatica 12 months previously, in spite of the fact that these rats still harboured living adult flukes from the sensitising infection. However, Hughes, Harness and Doy

(1977a) found that when rats with long standing primary infections were orally reinfected they regained their ability to resist a subsequent intraperitoneal implantation of adult flukes within 3 weeks, irrespective of whether or not the long-standing primary infection had been eliminated by an anthelmintic. The young flukes of the second infection were found to be alive and migrating in the liver parenchyma, whereas the implanted adult flukes were killed in the body cavity. Hence, they attributed the ability of rats to kill intraperitoneally implanted adult flukes to a recent migration by immature flukes through the liver parenchyma and maintained that rats in which flukes have reached the bile ducts progressively lose their ability to kill challenge flukes.

In spite of the considerable resistance stimulated against challenge by primary infections in rats, Hayes *et al.* (1972; 1973) and Goose and Macgregor (1973a) agree that primary infections are not affected by challenge infections. Also, Goose and Macgregor's (1973a; b) finding that anthelmintic treatment does not interfere with the ability of rats to resist reinfection was confirmed by Armour and Dargie (1974). The latter authors infected rats with 20 metacercariae each of *F. hepatica*, treated them with diamphenthide after 8 weeks and then challenged them with 20 metacercariae per rat 2 weeks after treatment. At necropsy, 10 weeks after challenge, a highly significant difference was found between the number of flukes recovered from the previously infected rats (2.6 ± 0.2) and the controls (5.5 ± 0.4), indicating a high level of

resistance to challenge due to previous infection.

Rats which can relatively quickly eliminate their fluke burdens retain their ability to resist re-infection (Hughes, Harness and Doy, 1976). These workers found that when rats of the Piebald Virol Glaxo (PVG) strain, which had lost their natural burden, 7 to 8 months after infection were challenged with 20 cysts of F. hepatica, killed and examined 4 months after challenge, they were found to have been markedly resistant to challenge as compared with controls.

The work of Rajasekariah and Howell (1978) further supports the conclusion that in rats primary infection initiates a high degree of resistance to re-infection. They reported that a primary infection with 5 metacercariae conferred a significant degree of resistance to challenge with 30 metacercariae per os. However, previously infected rats, still succumbed to intraperitoneal challenge with metacercariae of F. hepatica and there was no significant difference in the mean recovery from rats previously infected with 5 metacercariae and intraperitoneally challenged with 30 metacercariae as compared with controls.

2.2.1.2. Rabbits

Healy (1955) investigated the effect of repeated infections on the resistance of rabbits to F. hepatica. He found no difference in worm recoveries from previously infected rabbits and controls 60 days after challenge, although there was a reduction in the size of the flukes recovered 35 days after challenge from the hepatic parenchyma of previously infected rabbits. The secondary infection had no effect on the

primary one. In contrast, Ruther (1963) found that only 7% of the metacercariae of a challenge infection developed in rabbits with 2 or more previous infections whereas 46% developed in controls. He also noted a reduction in size of more than 65% in flukes recovered from previously infected rabbits as compared with flukes recovered from a single infection. When Ross (1966a) challenged 4 rabbits with 50 metacercariae each, 24 weeks after a primary infection with 75 metacercariae, he also found a reduction in the number and size of flukes recovered from the second infection. He reported much individual variation, to which he attributed the widely differing results reported in attempts to vaccinate rabbits against fascioliasis.

The effect of an existing infection on a subsequent one in rabbits was also investigated by Kendall, Hebert, Parfitt and Peirce (1967) and Kendall (1967). These authors showed that fewer flukes were recovered from a challenge infection in previously infected hosts than from those which had received a single infection. They were not convinced, however, that this reduction was due to destruction of flukes from the second infection. Rather, they thought it more likely to be due to a reduction in their growth rate, resulting from competitive inhibition, that makes them difficult to find in the hepatic parenchyma.

Kendall and Sinclair (1971) confirmed that no resistance developed in rabbits which had experienced a primary infection when judged by the number of flukes recovered from the secondary infection. However removal of the primary

infection with hexachlorophane 2 days before the second infection, resulted in a significant reduction in the number of flukes recovered.

Conversely Bolbol (1975) found no reduction in the number of flukes recovered from rabbits challenged with 100 cysts 4 weeks after the removal of a single primary infection of either 100 or 500 cysts. However, a statistically significant reduction was obtained in the number of flukes recovered from rabbits which had received two similar previous infections and treatments before challenge.

Fortmeyer (1973) found that oral superinfection of rabbits previously infected by the same route may result in a significant reduction in both the number and size of flukes recovered from the challenge infection. However, when rabbits were superinfected intraperitoneally, the flukes were reduced in size but not in number. Furthermore, Fortmeyer (1974) reported that rabbits infected intraperitoneally with 20-30 cysts of F. hepatica and subsequently challenged orally with 50 cysts showed no resistance to the challenge infection when the interval between the primary infection and challenge was 6 weeks. However, when the interval was increased to 13-16 weeks a significant resistance was encountered.

The evidence for resistance in rabbits to reinfection with F. hepatica is therefore somewhat contradictory, but it is quite clear that the effect of such resistance, when it can be demonstrated, is never as great as this phenomenon in rats.

2.2.1.3. Mice

The studies of Lang (1967) have shown in mice that previous infection with F. hepatica stimulates resistance to subsequent infection. A single immunising infection of two metacercariae tested after 40 or 60 days by a similar challenge infection resulted in a significant reduction in the number of flukes recovered from the challenge. In a further experiment, two previous infections, each with 2 metacercariae separated by 60 days were also found to result in a significant reduction of the fluke burden from a challenge infection administered 40 days after the second immunising infection. The author also observed earlier migration of the worms of the challenge infection from the liver parenchyma to the bile ducts in resistant mice. Furthermore various host responses including total body weight, total and differential leucocyte counts, spleen weights, serological response and liver histopathology were found to indicate a much more rapid response in the resistant animals than in controls. The histopathology of the liver including the timing and degree of lymphocytic infiltration in resistant and control mice led the author to the conclusion that delayed (cellular) hypersensitivity was responsible for this resistance.

Lang (1968) compared the numbers of flukes recovered from a group of mice with one or two previous infections 25 days after challenge with those recovered from a similar group 40 days after challenge. There was no significant difference. In contrast, a significant difference was found between the

fluke burdens from either of these previously infected groups as compared with previously uninfected controls. However, no such difference was found between the burdens recovered from the previously infected mice or the controls 20 days after challenge. Hence, the author suggested that the factors responsible for the stimulation of resistance are effective between 20 and 25 days after challenge. Lang's work was, however, criticised by Dawes and Hughes (1970) on the grounds that he had not included results obtained from all the 56 mice which received two immunising infections followed by challenges and that the selection of groups of 12 mice for post-mortem examination 20 and 40 days after challenge could have influenced his results.

Harness, Doy and Hughes (1977a; b) reported that significantly more flukes reach the liver 2 days after challenge in mice sensitised with a primary infection of 2 metacercariae, which is terminated by an anthelmintic after 10 days or in mice with a 3-week sensitising infection of irradiated metacercariae than in controls. However, no difference was observed in the numbers of flukes recovered from the livers of sensitised or control mice after 2 weeks. When the sensitising metacercariae were allowed to develop to maturity before challenge, no such rapid migration to the liver by the flukes from the challenge infection was observed and at 2 weeks after challenge significantly fewer flukes were recovered from the livers of the sensitised mice than from the controls.

Thus, experimental evidence appears to indicate that previous infections generally stimulate resistance to challenge with F. hepatica in mice. However, Harness, Hughes and Doy (1976) considered this evidence to be equivocal. They reported that experimental infections of mice are usually limited to 2 metacercariae because of the danger of death from a larger infective dose. Hence, they concluded that it is difficult to assess an immune response in mice by comparing the number of flukes recovered from resistant and control groups.

2.2.1.4. Sheep

Sinclair (1962) infected sheep with 150 metacercariae on 4 occasions and observed that there was little to indicate that previous infections induced any immunity which could prevent the successful development of subsequent infections. However, he observed delayed onset and reduced egg production by the adult flukes. The findings of Boray (1967a) also suggested that in repeated heavy infections, the tissue reaction caused by the migrating flukes may hinder the establishment of flukes from subsequent infections. Furthermore, sheep treated with an anthelmintic to eliminate a 5-week or 12-week primary infection were found to succumb to challenge with 200-4000 metacercariae, with no appreciable difference in the fluke burden, clinical symptoms or pathology between them and controls (Boray, 1967a; b).

Sinclair (1970) stated that, if sheep have any ability to acquire resistance against F. hepatica, the cellular reactions in the liver must be involved. To verify this

hypothesis, he investigated the development and pathogenicity of challenge infections with F. hepatica in lambs in which the inflammatory reaction to a previous infection had been suppressed by corticosteroids. In earlier studies he had demonstrated accelerated development and enhanced pathogenicity of F. hepatica in lambs which received daily injections of corticosteroids. The results of his later investigation convinced him that sheep with a previous infection do acquire a measure of resistance to a secondary infection. This resistance could be expressed by a reduction in the proportion of metacercariae from the challenge dose which reach maturity as well as by retardation during the early development of the flukes. Also, more rapid migration of flukes to the bile ducts may occur in resistant lambs resulting in earlier haematological changes. These findings are comparable to those of Lang (1967) in mice. According to Sinclair (1970) the cause of this rapid migration may be that the flukes enter the bile ducts to escape from the increasing tissue reaction in the host's liver parenchyma.

Another phenomenon observed by Sinclair (1970) was that the flukes of the challenge infection grew to a larger size in the corticosteroid treated lambs. He attributed this to the suppressive effect of corticosteroids on the development of biliary hyperplasia, which resulted in a more favourable environment for the flukes.

The role of the migrating immature flukes per se in stimulating resistance against a challenge infection was investigated by Sinclair (1971a). He used emetine hydrochloride

to eliminate a 4-week-old primary infection of 300 metacercariae in sheep. Six weeks later he challenged them and controls with 600 metacercariae. Contrary to his findings in 1970, he now observed delayed onset of anaemia and a later appearance of haemorrhagic lesions in the livers of the reinfected sheep, which led him to the conclusion that the flukes remained longer in the parenchyma than in controls. No significant difference was encountered between the recoveries from the challenged and control groups but the development of the challenge flukes was retarded. This retardation, occurring between the 6th and 10th weeks after challenge, was taken to be the expression of the measure of resistance initiated by the activity of the migrating immature flukes. Confirmation of these results came from Sinclair's (1973) studies in which he compared the development and pathogenicity of F. hepatica in three groups of sheep. One group had been treated with diamphenethide to eliminate a nine-week-old primary infection, one had received five previous infections each terminated after one week and one was previously uninfected. Temporary retardation of the development of the flukes derived from the challenge infections with delayed entry into the bile ducts resulted in both previously infected groups. As in the experiments in 1971a, no evidence was found of a reduced fluke burden in the previously infected sheep. Two of the findings led the author to suggest that an immunological type of response occurs in sheep after reinfection with F. hepatica. The first of these was the retardation of the

development of the flukes, particularly in the group of sheep which had received 5 previous weekly infections, in spite of the fact that the hepatic damage was not thought to be extensive at the time of challenge. The second was that gross examination of the livers of the challenged groups revealed a less severe reaction than in the controls. However, the effect of this immunological response on the flukes from the challenge infection was slight, being mainly expressed as a temporary retardation of development. Furthermore, the author did not exclude the possibility of an additional effect due to structural changes, provoked in the liver by the previous infections, acting as a physical barrier to the migration of the flukes derived from challenge and in particular to their entrance into the bile ducts.

Results and conclusions consistent with those of Sinclair (1971a; 1973) were also reported by Rushton (1977) who carried out his experiments in three groups of sheep. One group was superinfected with 400 metacercariae 11 weeks after a primary infection of 400 metacercariae, another group was challenged with 400 metacercariae one week after the elimination of a 10-week primary infection of 400 metacercariae and a control group which received only a single infection of 400 metacercariae.

Sinclair (1975) further examined the effects of previous infections in sheep by determining the levels of circulating eosinophils, plasma proteins, plasma glutamatic dehydrogenase, voluntary dry matter intake and plasma loss in the faeces in three groups of sheep. One group was previously uninfected,

one with 5 previous infections, each terminated after one week and one with a primary infection terminated after 15 weeks. He found no evidence to indicate resistance to the pathophysiological effects of the infection in either of the challenged groups. He considered this to confirm his previous conclusion of the occurrence only of an immunological type of response which is ineffective in protecting the repeatedly infected sheep.

Hence, as in rabbits, the ability of sheep to acquire resistance to reinfection with F. hepatica is controversial.

2.2.1.5. Cattle

The ability of cattle to acquire resistance to reinfection with F. hepatica has been demonstrated by many workers. Ross (1966b, 1967b) found that in calves initially infected with 200 metacercariae and then reinfected with 300 metacercariae 3 weeks later, there was no significant reduction in the numbers of flukes recovered. However, a significantly reduced total fluke burden was encountered when the challenge infection was administered 18 weeks after the primary infection. He referred to this penomenon as an "acquired self cure".

Elimination of a primary infection with an anthelmintic does not interfere with the ability of cattle to acquire resistance to reinfection (Boray 1967a). This author initially infected steers with 1000 metacercariae, treated them with hexachlorophene between the 14th and 19th week, and reinfected both them and controls with 5000 metacercariae 3 weeks after treatment. The steers were slaughtered 22 weeks after challenge and a reduction of about 78% in the fluke burden from

the previously infected steers, as compared with controls was found. Previously infected animals also showed fewer clinical signs, lower egg output and a short patency in the challenge infections. These findings were confirmed by Doyle (1971, 1973). In 1971, this author found that there was no significant difference in the number of flukes recovered from calves infected with 750 metacercariae and superinfected with 1650 metacercariae after 17 weeks, whether or not the primary infection had been eliminated with nitroxynil one week before challenge. All the calves were killed 13 weeks after challenge. The treated and reinfected calves contained 88% fewer flukes than the controls.

Doyle (1973) demonstrated a relationship between the duration of the primary infection and resistance to challenge with F. hepatica. Furthermore, he suggested that resistance increases with the duration of the primary infection. He found no significant resistance to challenge in calves after a primary infection of 7 weeks. However, a significant reduction (72.5%) in the number of flukes in reinfected calves was encountered after a primary infection of 12 weeks. As previously mentioned, Boray (1967a) and Doyle (1971) both reported 77-88% resistance after primary infections of 14-19 weeks and 16 weeks respectively. Van Tiggele and Over (1975) also reported that after a 12-month primary infection some immunity developed against reinfection, whereas after a primary infection of 6 months there was only slower development of the flukes with no reduction in the numbers.

As Doyle (1973) found no resistance to challenge with F. hepatica in calves after a primary infection of 7 weeks in spite of the evidence of fibrosis after this period (Dow, Ross and Todd, 1967), he excluded the possibility of the resistance being due to physical factors such as the degree of fibrosis or cholangitis and suggested that the resistance is dependant on acquired immunity in the host.

Armour, Dargie, Doyle, Murray, Robinson and Rushton (1974) eliminated an 11-week-old primary infection of 750 metacercariae with an anthelmintic and then found that the treated cattle were still highly resistent to challenge with 1650 metacercariae as compared to controls. Also, Kendall, Sinclair, Everett and Parfitt (1978) terminated a 32-week primary infection of 1000 metacercariae of F. hepatica in two groups of calves and then challenged them with 1000 metacercariae at 3 and 22 weeks respectively. They found that the resistance to reinfection was independent of the continued presence of the primary infection and that the degree of resistance to challenge 3 weeks after the elimination of the primary infection was similar to that found after 22 weeks. The number of flukes recovered from both groups was reduced by 82% as compared with single-infection controls. There was also a reduction in the size of the flukes recovered from both reinfected groups.

2.2.2. Stimulation of resistance to Fasciola hepatica by infection with attenuated metacercariae.

The successful vaccination of calves with X-irradiated larvae of Dictyocaulus viviparus against parasitic bronchitis

(Jarrett, Jennings, Martin, Macintyre, Mulligan, Sharp and Urquhart, 1958) initiated many attempts to find a similar way to vaccinate against fascioliasis. These attempts depended on achieving attenuation of metacercariae by ionising radiation with X- or gamma-rays so that the pathogenicity of the flukes which emerge from them is reduced buttheir ability to initiate protective host response, identified by histological and serological methods, is maintained (Hughes, 1962a; b; Movesesijan and Cuperlovic, 1970; Sokolic, 1971).

The first report on attenuation of Fasciola was given by Jarrett, Jennings, Macintyre, Mulligan and Sharp (1959). Working with metacercariae of Fasciola gigantica they stated that 5 kiloroentgens (Kr) were sufficient for their inactivation. Many workers afterwards attenuated F. hepatica by X-irradiation of the metacercariae before administration into different hosts. Wikerhauser (1961a) reported reduced pathogenicity of F. hepatica in rabbits infected with metacercariae X-irradiated at levels ranging from 3-20 Kr. Also Hughes (1962b) found that X-irradiation of metacercariae of F. hepatica at levels from 4-100 Kr reduced the pathogenicity of metacercariae to such an extent that mice did not die 3 - 4 weeks after infection as they would if they had been infected with normal metacercariae. Hughes (1962b) found that the minimum level of X-irradiation which attenuates the flukes so that they do not kill mice was 2 - 4 Kr. Reduced pathogenicity of young flukes following X-irradiation of metacercariae was also reported in rats (Thorpe and Broome, 1962), rabbits and sheep (Hughes, 1963) and cattle (Boray, 1967a).

Sokolic (1968) suggested that a greater antigenic stimulation might result from the administration of irradiated rather than normal metacercariae because of the larger dose of the former which a host can withstand. However, attempts to vaccinate animals with such irradiated metacercariae produced conflicting results.

2.2.2.1. Rats

Thorpe and Broome (1962) vaccinated rats with single doses of metacercariae irradiated at levels ranging from 1-10 Kr and challenged them either 7 weeks or 11 weeks later. Vaccination with metacercariae irradiated at 2.5 Kr initiated a significant resistance with a mean reduction of 50% in the fluke burdens in rats challenged 7 or 11 weeks after vaccination. A significant resistance was also found in rats vaccinated with metacercariae exposed to 5 Kr or 7.5 Kr and challenged after 11 weeks. The mean fluke burden per rat from the vaccinated and challenged groups was 7.7 and 6.6 for animals challenged after 7 weeks and 11 weeks respectively. According to the authors if there had been no immunity the mean recovery per rat would have been 11.5 and 8 respectively. The latter figures were the sum of the mean recoveries from the un-challenged vaccinated control group and the challenge control group. These results, however, were criticised by Dawes and Hughes (1964) who considered that this procedure of summating fluke numbers from two control groups was of questionable validity.

On the other hand, the findings of Thorpe and Broome (1962) gained support from the work of Corba, Armour, Roberts and

Urquhart (1971), Armour and Dargie (1974) and Armour, Dargie, Doyle, Murray, Robinson and Rushton (1974). These authors vaccinated rats with 2 or 3 doses of 20 irradiated metacercariae (2.5 - 3.5 Kr) at weekly intervals and challenged them with 20 normal metacercariae. A highly significant resistance to challenge was found in vaccinated rats as compared with non-vaccinated controls.

2.2.2.2. Rabbits

All attempts to vaccinate rabbits with irradiated metacercariae of F. hepatica have produced negative results. Thus Hughes (1962b) used metacercariae X-irradiated with 2-4 Kr but no difference was found between the numbers of flukes recovered from vaccinated rabbits and from controls. A similar result was also obtained by Hughes (1963) when rabbits were given 2 doses of 500 irradiated metacercariae (4 Kr) at an interval of 3 weeks and challenged 4 weeks afterwards with 15 normal metacercariae.

2.2.2.3. Mice

Most attempts to immunise mice with irradiated metacercariae of F. hepatica also failed. Hughes (1962b) found no resistance to challenge in mice previously infected with metacercariae irradiated at 2-4 Kr. Furthermore, no evidence of resistance was found by Hughes (1963) in mice infected with two doses of either 20 or 40 irradiated metacercariae (3-4 Kr) at an interval of 3 weeks and challenged 3 weeks or 7 weeks later with 10 normal metacercariae.

Dawes (1963; 1964) repeated Hughes' (1963) experiments in mice and also found no evidence of resistance to challenge in terms of a reduction of fluke burden or delayed maturation, but he reported reduced growth by the flukes 3 - 4 weeks after challenge. Sokolic (1967) again found no significant resistance to challenge with normal cysts after previous infection with irradiated metacercariae in mice.

In contrast to these previous findings, Harness, Hughes and Doy (1976) reported that a protective mechanism may operate in mice vaccinated with irradiated metacercariae. Vaccination comprised either one oral dose of 20 metacercariae irradiated at 3.8 Kr or 2 such doses given at an interval of one week. Three weeks later both vaccinated mice and controls were challenged with 100 normal metacercariae. At autopsy 2 days after challenge the immature flukes were recovered from the peritoneal cavities of all mice. The fluke burdens from both vaccinated groups were significantly lower than those from controls. Thus Harness et al. concluded that a protective response had operated at the intestinal wall. However the results of a similar experiment (Harness, Doy and Hughes, 1977b) in which they killed the mice 12 or 14 days after challenge did not lend support to this conclusion as a similar number of flukes were recovered from both groups. This result indicated that the immature flukes migrated more quickly from the peritoneal cavity of mice with a previous infection with irradiated metacercariae than in controls. The authors therefore concluded that this rapid migration in the sensitised

mice could account for the reduced number of flukes recovered from the peritoneal cavity of such mice 2 days after challenge, as compared to controls.

2.2.2.4. Sheep

Vaccination of sheep with 2 doses of X-irradiated metacercariae (3 Kr) at intervals of 3 weeks failed to stimulate resistance to challenge with F. hepatica (Hughes, 1963). Boray (1967a) also found no evidence of an immune response in sheep previously infected with 3 doses of 1000 X-irradiated metacercariae (20 Kr) and challenged with 4000 normal metacercariae. However the onset of anaemia was delayed in the vaccinated sheep.

On the other hand, Dargie, Armour, Rushton and Murray (1974) carried out an experiment in which sheep were infected with 6 doses of 100 irradiated metacercariae (3 Kr) of F. hepatica before challenge with 750 metacercariae. A mean of 36 flukes was recovered from the vaccinated group compared to a mean of 105 flukes from non-vaccinated controls. Even this considerable difference in the fluke burdens was not statistically significant, but the authors considered it to be some evidence for the ability of sheep to acquire resistance against challenge with F. hepatica.

2.2.2.5. Cattle

Attempts to vaccinate cattle against fascioliasis with irradiated metacercariae gave more favourable results. Boray (1967a) infected calves with 3 doses of 3000 X-irradiated metacercariae (20 Kr) at intervals of 6 weeks and challenged them with 5000 normal metacercariae 3 weeks later. At

autopsy 16 weeks after challenge, there was no significant difference between the fluke burdens in the previously infected calves and in the controls. However the vaccinated calves showed fewer pathological changes in the liver.

Bitakaramire (1973) infected 7 calves with 2 doses of 1000 gamma-irradiated metacercariae of F. gigantica (3 Kr) at intervals of 6 weeks. These calves were then challenged together with controls 14 weeks after the second immunising dose. Twelve weeks after challenge, 5 of the vaccinated calves were found to be free of any Fasciola burden and did not show pathological changes in the liver. A fluke burden of 1 - 2 % of the challenge dose was recovered from the other two vaccinated calves, whereas the mean recovery from the non-vaccinated control group was 23.1% of the challenge. However, Armour and Dargie (1973) reported that when Armour, Dargie and Doyle (unpublished results) repeated Bitakaramire's (1973) experiment in calves using irradiated metacercariae of F. hepatica for vaccination, they found only 68% resistance in comparison to the 98% reported by Bitakaramire.

Armour, Dargie, Doyle, Murray, Robinson and Rushton (1974) vaccinated calves with 2 doses of 1000 metacercariae, irradiated at 3.5 Kr, at an interval of one month. Four and eight weeks after the second immunising dose two groups of calves were challenged with 850 normal metacercariae together with controls of the same age. A reduction of 30% in the fluke burden was found in the group challenged 4 weeks after immunisation compared with controls, whereas a reduction of 70% was found in the group challenged 8 weeks after immunisation. However, the

pathological changes were similar in the livers of both vaccinated and control calves.

Nansen (1975) reported that vaccination of calves with metacercariae of F. hepatica irradiated at 3 Kr may result in a significant resistance to natural infection. He vaccinated groups of calves with 3 doses of 1500 attenuated metacercariae at intervals of 4 weeks before they were turned out onto a fluke infected pasture for a grazing season of 24 weeks. Twelve weeks after the end of the grazing season, 71% fewer flukes were recovered from the vaccinated calves as compared with controls. Further evidence of resistance was also shown by the stable serum Y - glutamyltranspeptidase levels in the vaccinated calves in comparison with the elevated levels in non vaccinated controls, the enzyme being used as a measure of hepato-biliary damage caused by the flukes. From these results the author suggested that vaccination of calves with gamma-irradiated metacercariae could be an acceptable method of vaccination, since the hepatic pathological changes due to the vaccine do not greatly disturb liver function. However, he also pointed out an important deficiency in the vaccine, namely, the development of some vaccine-derived parasites to maturity. Nansen therefore called for further investigations to determine the appropriate dose of irradiation which prevents the maturation of flukes and to elucidate whether resistance can be stimulated without mature flukes in the liver, as well as to define the appropriate vaccination dose and the interval between vaccination and challenge.

2.2.3. Stimulation of resistance to Fasciola hepatica with homologous somatic extracts.

The use of antigen preparations from whole flukes or fractions of crude fluke material for vaccination against fascioliasis have produced conflicting results in different hosts. Eriksen and Flagstad (1974a) and Lang (1976) reported that parenteral injection of whole fluke antigen did not produce resistance against fascioliasis in rats and mice respectively. However, Lang and Hall (1977) stimulated resistance in mice by vaccination with antigens prepared by sonic disruption of immature F. hepatica. Pooled homogenate of 16-day-old flukes was sonicated until all the cells were disrupted. It was then centrifuged and the supernatant used as antigen. This contained about 500 µg of protein/ml. Vaccination with one injection of the sonicated antigen stimulated resistance which resulted in an 86% reduction in worm burden. Two injections resulted in an 82% reduction but the pathological changes in the liver were severe. The authors therefore considered that two injections with the sonicated antigen may have produced hypersensitivity in the test animals.

Most attempts at vaccination were however, carried out in rabbits. Kerr and Petkovich (1935) gave nine 1 ml intra-peritoneal injections of a 1% suspension of dried flukes to each of seven rabbits at intervals of one or two days. Five weeks later, each rabbit was challenged with 13 metacercariae, together with 3 controls. At autopsy 13 - 17 weeks after infection, the mean number of flukes recovered from vaccinated

rabbits was 2.1 and that from controls was 8.3. It was also observed that the flukes were calcified in two of the vaccinated rabbits and that eggs did not appear in their faeces. According to these authors, this result indicated that it is possible to stimulate immunity by injections of dried fluke material. However, Urquhart, Mulligan and Jennings (1954) questioned the significance of these findings in view of the small number of control rabbits. They suggested that the reduction could be due to the normal variation in 'take'. Urquhart et al. (1954) also vaccinated two groups of 7 rabbits with an alum precipitated protein antigen (10 mg protein N/ml) prepared from crude adult fluke homogenate. This antigen was given intramuscularly at intervals of 8 - 10 days. One group received 3 injections and the other received 6. Both vaccinated groups and 11 control rabbits were challenged with 50 metacercariae of F. hepatica each. It was found that although high titres of precipitins were produced, vaccination with these fluke proteins had no influence on the number of F. hepatica which developed. However, determination of their total nitrogen content showed that there was retardation of development of the flukes recovered from the vaccinated rabbits. These authors therefore concluded that if the reduction previously reported by Kerr and Petkovich (1935) was real it might have been due to the influence of antigens other than the proteins.

The histopathological changes in the livers of rabbits vaccinated with fluke antigens were found to be less severe than

in the livers of unvaccinated controls by Shibanai, Tozawa, Takahashi and Isoda (1956). They vaccinated separate groups of rabbits intradermally, subcutaneously, intraperitoneally and orally and challenged them after 10 weeks together with controls. At post-mortem examination 8 weeks later, the livers of the rabbits which had received intraperitoneal or oral vaccinations showed lesions of medium severity. The lesions in the livers of rabbits vaccinated intradermally or subcutaneously were least severe whereas the un-vaccinated controls had the most severe lesions. However these authors were unsure whether the reduced severity of lesions in the livers of vaccinated rabbits was due to the vaccine.

Complete resistance to challenge was reported by Babadzhanov and Tukhmanynts (1958) in rabbits vaccinated with a whole F. hepatica extract which contained a considerable amount of polysaccharide but no proteins or lipids. These rabbits were vaccinated with 6 intravenous injections and challenged 2 weeks later with 10 metacercariae. However many other workers have found no evidence of immunity in rabbits vaccinated with whole fluke extracts. Healy (1955) vaccinated rabbits with such extracts and reported no evidence of resistance in animals autopsied 60 days after challenge, although retarded development of the flukes was found when the vaccinated rabbits were killed 35 days after challenge. Geyer (1967) made reference to his unpublished results which had indicated no evidence of immunity in rabbits vaccinated with 5 injections of materials from homogenized and lyophilized flukes containing proteins, carbohydrates and lipids. Antigens

prepared from lyophilized adult flukes were also used by Hughes (1963) in attempts to vaccinate rabbits against fascioliasis. In an experiment similar to that of Kerr and Pitkovich (1935) Hughes vaccinated rabbits with 9 intraperitoneal injections of the antigen before challenging them with 13 metacercariae each. At post-mortem examination 14 weeks later a mean of 1.7 flukes was recovered from vaccinated rabbits whereas a mean of 5.6 flukes was recovered from unvaccinated controls. However, no evidence of resistance was found when the experiment was again repeated. Ross (1967c) also found no evidence of resistance in rabbits vaccinated with 5 subcutaneous injections of 1 ml of whole fluke homogenate. However, 2 subcutaneous injections of 1 ml of homogenate containing 0.66g% protein prepared from immature (6-week-old) flukes at a 2-week interval resulted in retarded growth of flukes from a challenge infection between the 6th and 7th weeks after infection.

Attempts to vaccinate sheep with fluke antigen were also discouraging. Ershov (1959) used as a fluke antigen a complex containing about 60% polysaccharide and 30 to 40% albumen component. In a total of 97 vaccinated sheep, 10-25% were found to be solidly resistant to challenge, while the rest showed reduced susceptibility. However, vaccination resulted only in a short term immunity as no resistance was found in sheep infected 45 days after vaccination. The absence of any resistance to challenge was also reported by Hughes (1963) in sheep vaccinated with 9 intraperitoneal injections of adult fluke antigen over a period of 3 weeks. Similar results were

also reported by Ross (1967c) in a lamb and a calf vaccinated with 5 subcutaneous injections of 1 ml of whole fluke homogenate.

2.2.4. Stimulation of resistance to Fasciola hepatica by homologous metabolic products

The metabolic products of helminths have been used in attempts to stimulate immunity against many of these parasites (Soulsby, 1957a; Urquhart, Jarrett and Mulligan, 1962; Lehner, 1977). In fascioliasis, Healy (1955) attempted to vaccinate rabbits with pooled regurgitated caecal contents of adult flukes. No evidence of resistance to challenge was found when the vaccinated rabbits were killed after 60 days. However, retarded development of the flukes was found when the rabbits were killed after 35 days, while the flukes were still in the liver parenchyma. Purified caecal contents from adult F. hepatica were used by Lalic, Cuperlovic and Movsesijan (1976) to vaccinate rabbits against F. hepatica. Three injections of the vaccine (a total of 6 μg of protein) were given to each rabbit at 2-week intervals before challenge with 25 metacercariae. The antigen provoked an immune response and specific antibodies were found by precipitation, complement fixation and fluorescent antibody tests. At autopsy it was found that a mean of 16.8% of the infective dose developed in vaccinated rabbits whereas 4.8% developed in the non-vaccinated controls. Greater pathological changes were also found in the livers of vaccinated rabbits. The authors suggested that the immune mechanism provoked by the antigen may have influenced the susceptibility of the host.

Lang (1976) used metabolic products of 16-day-old F. hepatica maintained in Medium 199 for various periods of time as a vaccine against F. hepatica in mice. A single injection of medium in which the flukes had been incubated for 4 hours (4-hour incubate antigen) or 24-hours (24-hour incubate antigen) and which contained 160 ug or 480 - 500 μg protein respectively, failed to stimulate resistance against challenge, although host mortality was significantly lowered. On the other hand, four sequential vaccinations with 24-hour incubate antigen (520 - 560 ug protein) reduced the fluke burden by a mean of 83.3%.

Lang and Hall (1977) attempted to determine the time at which these protective antigens are produced by 16-day-old F. hepatica in vitro. They vaccinated groups of mice with incubate antigens produced over various periods of time. The highest resistance (86%) was shown by the group vaccinated with 12 or 24 hours incubate antigens. The authors therefore suggested that qualitatively different protective antigens are produced between 12 and 24 hours. Contrary to the previous report of Lang (1976) this later work suggested that sequential injections were not necessary for successful vaccination.

Lang's (1976) findings that resistance to F. hepatica can be stimulated in mice by sequential vaccination with 24-hours incubate antigens were not supported by Lehner (1977). The latter author carried out a similar experiment and obtained results in marked contrast to those reported by Lang. Lehner found no difference between vaccinated mice and controls in

infectivity rate, mortality rate or fluke burden. He also vaccinated rabbits and rats with metabolic antigens from adult and immature F. hepatica. Metabolic antigen for vaccination of rabbits was prepared by maintaining flukes recovered from rabbits in a continuous flow recirculating system containing Medium 199 for 7 days. The antigen used contained 2 ug/ml protein. Rabbits were vaccinated 3 times at intervals of 4 weeks and challenged with 100 metacercariae each, as were unvaccinated controls. Antibodies similar in some features to those in the serum of rabbits with a patent fluke infection were detected in the serum of vaccinated rabbits. However, at post-mortem examination, 8 weeks after challenge, no significant difference was found in the fluke burdens recovered from vaccinated or unvaccinated rabbits. Nonetheless, the flukes recovered from the vaccinated rabbits were significantly smaller than those recovered from the controls.

Similar experiments were also carried out by Lehner (1977) in rats using antigens from adult and immature flukes. Although the vaccinated rats also produced antibodies with some features in common with those in the serum of animals with patent infections, clear differences were also evident, as it was found that the vaccinated rats did not respond well to the non-protein component of the metabolic antigen, whereas infected animals showed a strong reaction with this component. In no case was any evidence of resistance to challenge found in vaccinated rats.

2.2.5. Stimulation of resistance to Fasciola hepatica by implantation of living flukes.

As protective immunity has generally been found to be best stimulated by living parasites, it was thought that the antigens responsible for this immunity may be released by the parasites during their development in the host (Rickard and Bell, 1971 a; b; c). Many authors have tried to stimulate immunity to infection with various helminths by implanting the particular parasite in a site different from its normal location in the host, so that it can release antigenic metabolic products without causing significant pathological changes. Rickard and Bell (1971b; c) excluded any possible effect from direct contact between the implanted parasite and the host by enclosing the implanted parasites in membrane diffusion chambers. They reported that activated embryos of Taenia taeniaformis and Taenia ovis contained in such chambers and implanted intraperitoneally into rats and lambs respectively produced diffusible 'functional' antigens that stimulated a high degree of resistance to subsequent challenge. This technique has not previously been used in fascioliasis, but direct implantation of flukes has proved to be a useful technique for immunological studies in this disease.

Ross (1967c) implanted 6-week-old flukes into the gluteal muscles of 6 rabbits, a lamb and a calf which had been given 5 subcutaneous injections of whole fluke homogenate over 4 weeks. Two weeks later they were challenged,

together with a group of animals which had also received the whole fluke homogenate but not the implant and a control group which had received neither homogenate nor the implant. At post-mortem examination after 8 weeks both vaccinated groups contained smaller numbers of flukes than the controls, but the difference was not conventionally significant. Ross also implanted a group of sheep in the intercostal muscles with 6-week-old flukes and similarly implanted another group twice at an interval of 3 weeks. Both groups were challenged with 100 metacercariae of F. hepatica together with controls. At post-mortem examination 10 weeks after challenge there was no significant difference in the lengths of flukes recovered from implanted and control groups. Once again both implanted groups contained fewer flukes than the control group, but this difference was also not conventionally significant. However, the author considered that the retardation of fluke development following injections of flukes extracts and the reduction in the numbers of flukes recovered from vaccinated groups, though conventionally not significant, probably indicated the involvement of acquired immunity.

Hughes and Harness (1972) transferred adult flukes from the bile ducts of rats, rabbits and goats into the peritoneal cavities of rats and rabbits to determine the survival time in the host, to assess the adaptation of the parasite to the host and to see the effect of maintaining flukes in vitro on their development after transfer into a new host. Adult flukes implanted in the peritoneal cavities of rats showed

no predilection for the liver but were found encapsulated in fibrous vascular cysts. As these cysts did not form around dead flukes, the authors suggested that they may be a host response to the presence of living flukes serving to prevent more widespread trauma. Flukes survived within these cysts for at least 6-7 weeks. They appeared to thrive well and laid eggs normally. However, those implanted into the intraperitoneal cavities of rabbits did not thrive, were reduced in size and either had no eggs or abnormal eggs in their uteri. The authors suggested that this could be attributed to the inadequate nature of the nutrition available in the body cavity of the rabbits and to the inability of the fluke to adapt to this host. This work was taken as a baseline for a subsequent experiment by Hughes and Harness (1973a) designed to study whether there are shared antigens between F. hepatica and its hosts and, if so, the significance of this for the adaptation of the fluke to the host. Such a 'host-antigen' effect had been reported by Smithers, Terry and Hockley (1969) with another trematode, Schistosoma mansoni. These workers showed that schistosomes taken from mice and implanted into monkeys, which had been sensitised against mouse tissue, were soon killed and that the response in the "anti-mouse" monkeys was highly specific for schistosomes derived from mice. Hughes and Harness (1973a) used either erythrocytes or liver and spleen tissues mixed with Freund's complete adjuvant to immunise rats and rabbits against goats and against each other. However, they could not detect any harmful effects on adult flukes implanted into a recipient

host which had been immunised against the donor's tissue. Hence, under the conditions of this experiment, they found no evidence of any 'host-antigen' associated with F. hepatica. However, in a later paper (1973b) these authors stated that this failure to demonstrate any harmful 'host-antigen' effect might be due to the large size of the adult flukes as well as to their unusual site away from the liver, as this might make them less accessible to immunological attack. To exclude such factors they transferred young flukes into the intraperitoneal cavities of rats immunised against the donor host's tissues. These transferred immature flukes showed a predilection for the liver and became sexually mature in the bile ducts, but they were not affected by any harmful 'host-antigen' effect. Furthermore, the results of a mixed agglutination test did not demonstrate any shared antigens. However, these findings, were not considered by the authors as conclusive proof of the absence of shared antigens between F. hepatica and its hosts, but rather as indicating that if such antigens occur they are either not accessible surface or tegument antigens or are not similar to erythrocyte antigens.

Lang (1974a) observed that F. hepatica incubated in immune homologous serum for 4 hours and then transferred into the peritoneal cavity of mice, migrated into the bile ducts at an earlier time than flukes incubated in normal serum. He suggested that the development of the host immune response during the parenchymal migration phase initiates rapid movement of the flukes into the bile ducts, where they are protected from

most of the host's immune responses. According to this author, such a response may explain why the 'host-antigen' effect could not be demonstrated with F. hepatica by Hughes and Harness (1973a; b).

Hughes, Treacher and Harness (1973) reported a new technique whereby either adult or immature flukes can be transferred into the gall-bladder of a recepient host. The adult flukes were found to remain and produce eggs in the gall-bladder while the immature flukes migrated into the bile ducts, produced hyperplasia and became sexually mature. Hughes and Harness (1974) used this technique to stimulate immunity to F. hepatica in cattle. Their preliminary results showed that immature flukes from mice implanted into calves reached sexual maturity and produced calcification of the bile ducts. The calves were then challenged orally with F. hepatica metacercariae but the final results were not reported in this paper as the experiment was still in progress.

Flagstad and Eriksen (1974) examined the survival and activity of adult flukes from cattle, sheep and goats in the subcutis of albino rats. They found that the flukes became encysted one or two days after transfer and that 2% of the flukes were still alive after 15 weeks. Eriksen and Flagstad (1974b) stimulated immunity in rats using this technique. They implanted rats subcutaneously with adult flukes from sheep and goats and challenged them orally after 3 weeks together with controls. At post-mortem examination, 3 months after implantation, 6% of the transferred flukes were found alive within

cysts. Subcutaneously implanted rats showed 50% resistance against oral challenge with metacercariae as compared to normal rats. The authors considered that the involvement of an immunological mechanism against the challenge infection was supported by the lack of fibrosis in the livers of implanted rats. Furthermore, they suggested that the antigens associated with this immunity might be in the form of a metabolic product from the implanted flukes. The possibility of a role for the eggs produced by the implanted flukes in this immunity was however not excluded.

Anderson, Hughes and Harness (1975) also transferred adult flukes into the subcutaneous tissues of rats to study the possibility of stimulating immunity against challenge when the hepatic migration stage is by-passed. Rats were implanted subcutaneously with one adult fluke and challenged orally with 20 metacercariae 12 days later. The implanted flukes became surrounded with a fibrous cyst. Flukes were found alive within these cysts 34 days after transfer, though their testicular activity had decreased by that time. Histological changes characteristic of cell mediated immunity and of antibody production were found in the lymph node draining the site of implantation whereas the contralateral lymph node remained non-reactive. An average 8-fold difference in weight was found between the draining and contralateral lymph nodes. Injection with adult fluke antigen produced evidence of intradermal hypersensitivity and circulating precipitating antibodies were present in the implanted rats, thus supporting the

histological evidence for antibody production. According to these authors, this finding demonstrated a state of hypersensitivity concomitant with antibody production induced by F. hepatica in the rat. The protective value of this hypersensitivity and antibody production was proved by a significant reduction in the fluke burden (34%) recovered from the implanted rats as compared with controls.

Hughes, Anderson and Harness (1976) reported that sensitisation of different hosts by subcutaneous implantation of adult flukes involves antigens in the metabolic products as well as the eggs of the adult flukes, whereas sensitisation by an oral infection involves antigens from all stages of the parasite. These authors compared the two routes of sensitisation in rats and reported that, when they were sensitised by subcutaneous implantation of adult flukes and subsequently challenged by implanting adult flukes in the peritoneal cavity, 23% of the challenge flukes were killed. On the other hand, 71% of adult flukes intraperitoneally implanted as a challenge infection were killed in rats sensitised by an oral infection. However, sensitisation by neither of the two routes proved effective against subcutaneously implanted adult flukes. Histological studies showed that about half the dead flukes had been rapidly killed after transfer and were surrounded by mononuclear cells. The authors considered that the rapidity with which these flukes were killed indicated the involvement of antibodies. However, the rest of the dead flukes were surrounded by neutrophils and it was suggested that although the

death of these flukes was probably also mediated by antibody, the rate of the response was sufficiently slow to allow the formation of sufficient antibody-antigen complex to give rise to the chemotactic response that attracted the neutrophils which could digest the dead flukes.

The role of different developmental stages of the fluke in stimulating resistance to F. hepatica was investigated by Rajasekariah and Howell (1978). They sensitised groups of rats by subcutaneous implantation of either metacercariae of F. hepatica, 4-week-old immature flukes, adult flukes or fluke eggs and challenged each group after 2 weeks. The resulting fluke burdens indicated that implantation of all the stages, except the adult worms, stimulated a significant degree of resistance against oral challenge. Implanted adult flukes were incapable of stimulating such resistance even though the period of sensitisation before challenge was extended and flukes from different donors (cattle and sheep as well as rats) were used. It was found that implanted metacercariae were able to excyst and migrate from the implantation site, to reach the liver and cause hepatic damage. On the other hand, although 4-week-old worms and eggs stimulated immunity they were not found to cause any liver damage. On the basis of these results, the authors suggested that the role of liver damage in the stimulation of resistance is not of major importance, at least in rats. The finding that implanted adult flukes are incapable of stimulating resistance to challenge in recepients is contrary to the previous results reported by Eriksen and Flagstad (1974b) and

Anderson et al. (1975). Rajasekariah and Howell suggested that this discrepancy might be because they used a different strain of rats in their experiment. They also suggested that the stimulation of resistance by implanted eggs but not by eggs laid by implanted adult flukes could be explained by the fact that the former are more widely dessiminated, as the latter become encapsulated and confined within a cyst.

Several authors have studied the results of implanting F. hepatica in mice. Thus Gold and Lengy (1972) investigated the migratory behaviour, survival time and the immunising effect of implanted adult F. hepatica in this host. The flukes were obtained from rabbits and guinea pigs and implanted into the intraperitoneal cavities of 20 mice. These mice were orally challenged after 10 days with metacercariae, as were 15 controls. Eleven mice died before challenge but 6 survived for at least 30 days after challenge. The percentage of fluke recovery from implanted mice and controls was 11.1% and 12.7% respectively thus indicating that the implanted adult flukes had failed to stimulate resistance to challenge. Most mice implanted with 1 or 2 flukes survived for at least 10 days, those implanted with 3 or 4 adult flukes died on the following day. Implanted adult flukes were generally found to be capable of extensive migration and some even reached the liver, although they were not able to 'relocate' within the bile ducts. Two implanted flukes survived for 25 and 40 days respectively in mice without apparent harm.

An attempt to determine the age of F. hepatica responsible for inducing acquired immunity in the mouse was made by Lang and Dronen (1972). They inserted 8 - and 16 - day-old flukes into the intraperitoneal cavities of mice. Both groups of flukes reached the liver after 3 days and the bile ducts when they were 32 days old. When the transferred flukes reached the age of 40 days, the implanted mice were orally challenged with 2 metacercariae per mouse and post-mortem examination was carried out 25 days later. Significantly fewer flukes were recovered from groups of mice immunised with 8 - or 16-day-old flukes than from unimplanted controls.

Although the 8-day-old flukes were present in the hepatic parenchyma for 21 days, while the 16-day-old flukes were only there for 13 days, the pathological changes caused in the liver by the latter were greater. The authors attributed these changes to substances produced by immature flukes which are antigenic and toxic to the hepatic tissue and suggested that the 16-day-old flukes produced more of these toxic products than the eight-day-old flukes, resulting in both the increased liver damage and the enhanced stimulation of the protective immune response.

Lang (1974b) transferred 12, 14, 18, 20 and 24-day-old flukes into mice and reported that they all reached the bile ducts when they were 30 to 32 days old. When the transferred flukes reached an age of 40 days he challenged each of the implanted mice orally with 2 metacercariae. At necropsy 25 days after challenge mice implanted with 12-, 14-, 16- or 18-day-

old flukes showed a significant resistance to challenge, whereas mice implanted with 20- or 24-day-old flukes did not. On the basis of these results the author suggested that the critical factor in the stimulation of immunity is the duration of parenchymal migration by young flukes and not their age. As no resistance was stimulated when hepatic migration of transferred flukes was shorter than 8 days, while a significant resistance was stimulated by 18-day-old flukes which migrated in the liver for 10 to 11 days, Lang suggested that a parenchymal migration of at least 10 days is a prerequisite for the stimulation of acquired immunity in mice.

Lang (1974a) later carried out further studies to investigate the importance of the age of the transferred flukes in the stimulation of resistance in mice. He incubated 12, 16, 18, 20 and 24 day-old flukes at 37.5^{o}C for 4 hours in immune serum from mice and transferred them into the peritoneal cavity of recepient mice. The latter were necropsied when the transferred flukes reached an age of 34 days. A significant reduction in the number of flukes recovered from these mice was found when compared to those from mice implanted with flukes incubated in heat-inactivated immune serum, normal serum, Medium 199 or buffered saline. No significant differences were observed in the number of flukes recovered from any of the former control groups. Since 12-24-day-old flukes were debilitated by immune serum and it had been previously shown that 12-18-day-old flukes were capable of stimulating resistance (Lang and Dronen, 1972; Lang, 1974b) it was concluded that the duration of parenchymal migration is of greater importance

in the stimulation of resistance than the fluke's age.

2.2.6. Passive Transfer of immunity to *Fasciola hepatica* by serum.

The efforts to elucidate the significance and mechanism of resistance to fascioliasis have included attempts at passive transfer of immunity by immune serum.

2.2.6.1. Rats

Armour, Corba, Dargie and Urquhart (1971) found that serum from rats with an immature infection failed to protect recepient rats from challenge with metacercariae of *F. hepatica*. Also the intraperitoneal transfer of immune serum from donor rats with a 10-week-old mature infection failed to confer resistance in recepient rats weighing 120-180 g, when each received about 4 ml of immune serum. Later however, Dargie (1973) and Armour and Dargie (1973; 1974) showed that rats can be immunised with homologous or heterologous immune sera. They injected 91 rats intraperitoneally, with 10 ml each of immune serum from donor rats, cattle or sheep infected with *F. hepatica*, both at the time of challenge with 20 metacercariae and again 2 days later. An overall reduction of 67% (mean 1.7 flukes) was found in the number of parasites recovered from these rats as compared with rats which received no serum or which received serum from un-infected donors (mean 5.1 flukes). A direct relationship was observed between the volume of immune serum transferred and the degree of protection obtained. Thus the mean number of flukes recovered from rats given 0, 5, 10 or 20 ml was 3.6, 2.0, 1.4 and 0.6 respectively. As immune serum transfer was found to be

protective if given within the first two days of challenge, but not if given 2 weeks before or 2 weeks after challenge, the authors concluded that immune serum is only protective against young flukes before their entry into the liver.

Antibodies of various immunoglobulin classes and subclasses differ in their biological activities and under certain circumstances an immune response can be restricted to one or another subclass (Spiegelberg, 1974). Armour and Dargie (1973) in a preliminary attempt to determine the immunoglobulin class involved in the transfer of passive immunity in rats at the time of challenge, transferred only the fraction IgG. A significant protection of 55% was found in the immunised rats as compared with controls. Such a protection was not found with IgM. Further work using a fraction of immune serum from rats and cattle which conferred 68 - 73% resistance to rats also indicated that IgG was a prerequisite for successful protection (Dargie, Armour and Urquhart, 1973).

Hayes, Bailer and Mitrovic (1974b) found similar results when they investigated the immunising effect of transferring homologous immune serum into rats at 0 and 14 days after challenge. Immune serum was obtained from rats infected with F. hepatica 7 - 8 weeks previously. Recepient rats had a mean weight of 64 g and were given intraperitoneal injection of 5 ml of serum and challenged with 10 metacercariae. At post-mortem examination 4 weeks later 66% fewer flukes were recovered from rats injected with normal serum at day 0, and 73% fewer flukes than from rats which

received no serum. These differences were highly significant while the difference between the two control groups was not significant. Furthermore, no significant difference was found between the number of flukes recovered from rats injected with immune serum 14 days after challenge and controls injected at a similar time with normal serum or controls which received no serum. However, a significant difference in fluke burden was found between the group of rats injected with immune serum at day 0 and that injected on day 14. Hence, the authors concluded that resistance due to immune serum is expressed against flukes younger than 14 days and that humoral antibodies are at least partially responsible for this resistance. Hayes, Bailer and Mitrovic (1974c) confirmed their earlier (1974b) results and reported evidence of resistance to F. hepatica due to immune serum transfer in two strains of rats, Fisher 344 and Sprague Dawley, thus indicating that this resistance was not limited to one strain. They found that volumes of 5, 2.5 or 1 ml of immune serum all gave a similar protective effect which was higher than that conferred by a volume of 0.2 ml. However even the rats which received only 0.2 ml of serum had significantly fewer flukes than controls which received no serum. The best results were obtained when serum was administered at the time of infection. Serum given 2, 4, 6 or 8 days after infection did not result in significant resistance, although there appeared to be a slight protective effect following transfer 2 or 4 days after infection. Immune serum from rats with a more chronic infection of 25 weeks was

found to have no protective effect. The protective effect could be eliminated by heating the serum for 30 minutes at 56°C and was also absorbed by live or dead F. hepatica. As most of the flukes recovered from immunised rats were the same size or were slightly larger than those from the controls, the authors suggested that immune serum has an 'all-or-nothing effect' against individual young F. hepatica.

Howell, Sandeman and Rajasekariah (1977) carried out in vivo and in vitro studies of the effects of immune rat serum on F. hepatica. Their results confirmed the previous findings that immune serum confers resistance in recepient rats. They used three batches of immune serum from two strains of rats. The first batch was collected from inbred DA rats which had been given a primary infection of 5 metacercariae at 5 weeks of age, a second infection with 30 metacercariae after 7 weeks and exanguinated 8 weeks later. The second batch was collected from Wistar rats in the same way except that they were exanguinated 1 week after the second infection. The third batch was similarly collected from Wistar rats but the levels of primary and secondary infections were 10 and 20 metacercariae respectively. The recepient rats were injected intraperitoneally with immune serum varying from 10 ml immediately before and after infection to 5 ml immediately after infection. In all cases a significant degree of protection was achieved. As no significant difference was found between the number of flukes recovered after injection with normal serum from 13-20 week old rats, and that from

rats which received no serum, the authors concluded that the factors involved in age resistance are not transmitted in serum.

The results of the *in vitro* studies of Howell *et al.* (1977) on the effects of immune serum on newly excysted flukes were similar to those of Wikerhauser (1961b). In both cases a precipitate was formed around newly excysted flukes in the presence of immune serum. This precipitate was considered to be made of one or more rat antibody-fluke antigen complexes. Howell *et al.* (1977) also found that although immune serum did not have a pronounced effect on the mortality of metacercariae *in vitro*, such metacercariae did not develop *in vivo*.

2.2.6.2. Rabbits and Guinea pigs

The phenomenon of precipitation which occurs around live larval stages of many nematodes and trematodes when they are incubated in immune serum has been used for the microscopic diagnosis of many of these infections e.g. trichinosis (Roth, 1941), ascariasis (Soresco and Panaitesco, 1958), metastrongylosis (Becht, 1960) and schistosomiasis (Papirmeister and Bang, 1948). However, the role of such precipitation in immunity has been controversial. Some workers have reported that it has an inhibitory effect on the young stages of parasites. Thus Sarles (1938) observed this effect on the larvae of *Nippostrongylus muris*, Otto (1940) on those of *Ancylostoma caninum*, Gonzales (1940) on those of *Trichinella spiralis* and Papirmeister and Bang (1948) and Standen (1952)

on the cercariae of Schistosoma mansoni. On the other hand, Roth (1941) found no such an effect on the larval of T. spiralis. Precipitation was reported by Wikerhauser (1961b) around excysted metacercariae of F. hepatica when these were incubated in immune rabbit or bovine sera. According to this author, this precipitation was a sensitive and specific test for the immunological diagnosis of fascioliasis in these animals. Sewell (1968) also found such precipitation when newly excysted flukes were incubated in immune rabbit, ovine or bovine serum. He reported that the antibodies involved in this precipitation were absorbed by none of the serologically active components in the metabolic products of F. hepatica.

To test the inhibitory effect of precipitating serum on F. hepatica in vivo, Wikerhauser (1961b) carried out the following experiments. Two groups, each of 3 guinea pigs (500 - 950 g body weight) were infected intraperitoneally with 5 excysted metacercariae that had been incubated in either immune or normal bovine serum at 37°C for 18 hours. The guinea pigs also received 1 ml of the corresponding serum intraperitoneally at the same time. 'A lighter' infection was found in the animals which had received metacercariae incubated in immune compared to those given metacercariae incubated in normal serum. The former guinea pigs also survived longer and had less severe changes in the liver and peritoneum. Similar results were obtained in another experiment in which each animal was simultaneously injected

intraperitoneally with 10 freshly excysted metacercariae and 2 ml of immune or normal bovine serum per kg body weight. In a further experiment in which guinea pigs were infected orally with 10 or 20 metacercariae and given 2 ml immune bovine serum subcutaneously or intraperitoneally per Kg body weight $\frac{1}{4}$ - 3 hours later, the immune serum had no effect. However the author pointed out that these results are only of relative significance because of the small number of animals in each group (3).

Baalway (1975) transferred either homologous immune serum from rabbits infected with F. gigantica or heterologous immune serum from infected goats into groups of 3 rabbits both at the same time and 2 days after challenge with metacercariae of F. gigantica. He found that resistance ranged from 25 to 40% after intraperitoneal transfer of 1 to 10 ml of concentrated homologous immune serum. The highest protection was found in rabbits which received 10 ml. The same dose of concentrated immune serum from infected goats also conferred the highest resistance (78%). The author concluded that for immune serum to be effective it must be in high concentration, in a sufficiently high dose and must be collected while the young flukes are still migrating in the peritoneum of the donor.

2.2.6.3. Mice

Hughes (1963) found that when mice which had received serum intraperitoneally from infected rabbits or from rabbits in which vaccination had been attempted were infected with 5 metacercariae together with controls, no difference was to be

found in the degree of infection or in the survival time between the two groups. These results support those of Wikerhauser (1961b) in guinea pigs. However, Ruther (1963) infected each of 10 mice with 5 metacercariae which had been incubated in immune rabbit serum at 37°C for 24 hours. Three weeks after infection the mean fluke recovery from these mice was 0.3 while that from controls infected with cysts incubated in normal rabbit serum was 3.0. A significant reduction in size was also observed. Further, as already mentioned, Lang (1974a) found that when 12-24-day-old flukes were incubated in homologous immune serum and transferred into recepient mice, the number of flukes recovered was reduced compared with that from mice implanted with flukes incubated in heat-inactivated immune serum, normal serum, Medium 199 or buffered saline. From this result and in particular since the effect of immune serum was significantly decreased by heat inactivation at 56°C for 30 minutes, the author suggested that complement was necessary for the antigen-antibody reaction involved in this type of resistance. However there was a significant reduction in the lengths of the flukes recovered from mice implanted with flukes incubated in inactivated immune serum as compared to those from mice implanted with flukes incubated in normal serum. This led the authors to suggest that this effect is not only due to IgG, as suggested by Dargie *et al.* (1973) but also to IgM. According to Lang, the IgM may combine with a cell membrane antigen to form a complex which can activate complement to produce immune lysis of the cells and death of the flukes.

Lang (1976) incubated F. hepatica in immune serum from mice which had been injected with fluke incubate antigen from 16-day-old flukes or in immune serum from mice with 25-day-infections. Such flukes were then transferred into recepient mice. At post-mortem examination it was found that in both cases the immune serum had had a debilitating effect, shown by a significant decrease in fluke recovery and host mortality. Serum from mice which had been injected with somatic antigen or from those with 100-day infection had no such debilitating effects. Hence, the author suggested that the antigens in the incubation medium which stimulate the production of antibodies harmful to the flukes are produced only by immature flukes. However, incubation of young flukes in antiserum from mice with mature infections was found to result in earlier migration after transfer. Therefore it was suggested that the mature flukes also produce the antigens but that their location in the bile ducts does not allow these antigens to have sufficient contact with the host to maintain immunogenic levels.

2.2.6.4. Sheep

Soulsby (1957b) described a thermostable component of normal sheep serum which has a lethal effect on the miracidia of F. hepatica in the presence of fresh guinea pig complement. Such activity was also found in sera of sheep infected with F. hepatica and was considered to be a natural property of the serum. The author concluded that this component is similar to others which have an antagonistic effect on a wide

range of helminths and which are present in normal sera from various animals. He suggested that such components should be determined so that their effects could be differentiated from manifestations of resistance.

Armour and Dargie (1973) reported that sheep could be protected against F. hepatica if large amounts of immune serum are used. It was found that 400 ml of immune serum injected intraperitoneally resulted in 80% protection against a challenge dose of 750 metacercariae.

2.2.6.5. Cattle

Armour, Corba, Dargie and Urquhart (1971) found that immune serum from a calf with an immature infection with F. hepatica failed to protect a recepient calf from challenge. A similar result was obtained by Corba, Armour, Roberts and Urquhart (1971) who transferred 2400 ml of immune serum from a donor calf with a mature F. hepatica infection into the peritoneal cavity of recepient simultaneously challenged with 1000 metacercariae. The comparison was made with a calf which received no serum. However, Armour and Dargie (1973) reported that cattle could be immunised if even larger volumes of immune serum were used. Administration of 3.6 litres into a 250 Kg bovine recepient resulted in 99% protection against challenge with 1000 metacercariae of F. hepatica compared to a control which did not receive serum.

2.2.7. Transfer of immunity to Fasciola hepatica by lymphoid cells.

2.2.7.1. Rats

Corba, Armour, Roberts and Urquhart (1971) investigated

the immunising effect of lymphocytes transferred into rats from infected donors. Suspensions of lymphoid cells were prepared from the spleens and lymph nodes of rats with an immature F. hepatica infection (4 weeks), mature infections (8 and 10 weeks) or vaccinated with 3 weekly doses of irradiated metacercariae. Lymphoid cells from the last two donors transferred intraperitoneally into syngeneic recepients conferred a significant resistance of 66.7% - 73.7% and 100% respectively. However, lymphoid cells from donor rats with immature infections failed to confer protection.

Although the successful transfer of immunity required lymphoid cells from donors with a minimum infection of 8 weeks the authors excluded the possibility that the parasite should be mature for the successful transfer of resistance. This is because the best protection was obtained by lymphoid cells from donors infected with irradiated metacercariae whose survival time does not exceed 3 or 4 weeks. As immune serum from these donors conferred no protection on recepients, the authors suggested that the immune reaction to F. hepatica could be cell-mediated, at least in the rat. These results were confirmed by Armour and Dargie (1974) who transferred viable lymphoid cells from donor rats with 10-week infections to syngeneic rats which were challenged at the time of transfer. A significant reduction in fluke burden was obtained in the immunised rats compared to controls which received either no cells or non-viable cells. This reduction was found to be related to the quantity and persistence of the antigenic stimulus in the donor. Thus the transfer of lymphoid cells

from donor rats with a mean burden of 7, 6 or 3 flukes resulted in reductions of 81%, 68% and of 30% respectively, the latter result not being significantly different from the controls.

2.2.7.2. Mice

Lang, Larsh, Weatherley and Goulson (1967) investigated the role of delayed (cellular) hypersensitivity in immunity to F. hepatica. They obtained peritoneal exudate cells from donor mice with 35-week infections and injected them intraperitoneally into recepient mice which were each challenged with 2 metacercariae 3 weeks later. At necropsy 40 days after challenge, there was a highly significant reduction in the number of flukes recovered from the immunised mice as compared with the untreated mice. More rapid responses were also observed in the vaccinated mice. These were manifested by an earlier loss of weight and earlier changes in the differential leucocyte counts compared to the controls. According to the authors these results together with previous histopathological findings in the liver (Lang, 1967) indicate that a specific delayed sensitivity reaction takes place and causes local tissue damage. This damage results in nonspecific allergic inflammation and biochemical changes which lead to the rapid migration of flukes to the bile ducts and may cause the death of some of the young flukes.

2.2.7.3. Sheep

Sinclair (1971b) investigated the role of lymphoid cells in resistance to F. hepatica in inbred sheep. He prepared homogenates of lymph nodes and spleen from donor sheep infected

with F. hepatica 8 weeks previously, as well as from uninfected control sheep. These homogenates were administered intravenously or intraperitoneally immediately before challenge. The number and size of flukes recovered as well as the liveweight, packed cell volume, eosinophil counts and faecal loss of Cr^{51} labelled plasma showed no evidence of resistance in the vaccinated group. In contrast, Dargie (1973) reported briefly that lymphoid cells from infected sheep can confer immunity on highly inbred recepients.

2.2.7.4. Cattle

Corba, Armour, Roberts and Urquhart (1971) transferred lymphoid cells from the hepatic lymph nodes of a calf which had received 2 injections 15 weeks apart of 1000 metacercariae into the peritoneal cavity of a monozygous twin calf. At post-mortem examination a significant reduction of 79.7% was found in the number of flukes recovered from the immunised calf as compared with the monozygous control. These results were confirmed by Dargie (1973).

2.2.8. The stimulation of resistance to Fasciola hepatica in sheep by infection with the metacestodes of Taenia hydatigena

This procedure was suggested as to be "new initiative' in the attempt to stimulate immunity to fascioliasis in the natural host (Campbell, Kelly, Townsend and Dineen, 1977). The proposal was based on the work of Dineen, Gregg, Windon, Donald and Kelly (1977) who found that vaccination of sheep with irradiated Trichostrongylus colubriformis larvae resulted

in a protection of 98-100% against the simultaneous challenge with infective larvae of the same species, or of a closely related species (T. vitrinus) and also of a generically unrelated nematode (Nematodirus spathiger). From these results, they concluded that 'vigorous immunological reaction to a helminth parasite can cause the simultaneous expulsion of other generically related and unrelated species 'and that it may not be essential for the target parasite to be antigenically related to the species used to promote the response'. Campbell et al. (1977) therefore tried to stimulate resistance to F. hepatica by previous infection with Taenia hydatigena (Cysticercus tenuicollis). The latter was chosen because of three characteristics. Firstly, it has a migratory pattern similar to that of F. hepatica in sheep; secondly, it is possible to stimulate a high level of resistance to homologous challenge and thirdly, it is of low pathogenicity in sheep.

All the sheep used in the experiment were first treated with levamisole to eliminate nematode infections before they were allocated to 5 groups of 8 animals. Each animal in 4 of the groups was orally infected with 1000 Taenia hydatigena eggs. Two of these groups were treated with mebendazole to eliminate the metacestodes either 3 or 12 weeks after infection, before intra-ruminal challenge with 400 metacercariae of F. hepatica. The other two groups were superinfected with a similar dose of F. hepatica either 3 or 12 weeks after infection with the cestode. The fifth group was a control for the challenge with F. hepatica. Fifteen weeks after challenge

high levels of resistance, namely 96% and 98% were found in the group infected with cysticerci for 12 weeks and in the group whose infection with cysticerci was removed after 12 weeks respectively. These two resistant groups also showed less hepatic fibrosis and their packed cell volumes were not reduced, as compared to the controls. On the other hand, there was no evidence of resistance in the two groups infected with the cestode for only 3 weeks. Half the number of sheep in each of the 5 groups was left alive for a further experiment described by Dineen, Kelly and Campbell (1978). These authors found that the group of sheep which resisted challenge with F. hepatica after a previous infection with T. hydatigena for 12 weeks maintained this resistance against another challenge 9 months later. However, the group of sheep in which the cestodes infection had been eliminated after 12 weeks were fully susceptible to the second challenge with F. hepatica after 9 months, even though they were resistant after 12 weeks. The two groups which were susceptible to challenge with F. hepatica after a 3-week infection with T. hydatigena, were also susceptible to the second challenge with F. hepatica after 9 months. The observations that the maintenance of resistance against F. hepatica was dependent on the persistence of infections with the metacestodes and that this resistance was maintained by the cysticerci in a site remote from the reactive tissue, led the authors to suggest that infection with T. hydatigena stimulates an immunological mechanism rather than a physical barrier against F. hepatica. Furthermore, the observation

that the sheep which had been susceptible to challenge with F. hepatica after a 3-week infection with the cysticerci were also susceptible to the second challenge 9 months later, led them to suggest that the first challenge with F. hepatica prevented the development of cross-protection because it destroyed the cysticerci before they were able to initiate resistance against Fasciola. Alternatively, fascioliasis could be immunosuppressive. According to the authors the latter suggestion may explain why the group of sheep which acted as a control for challenge with F. hepatica succumbed to rechallenge after 9 months.

CHAPTER 3

MATERIALS AND METHODS

3.1. Production of infective material

The need for a constant supply of metacercariae of F. hepatica as infective material throughout the course of the work necessitated the maintenance of the intermediate host, Lymnaea truncatula (Müller) in the laboratory. Snails were obtained from the stock colony at the Department of Tropical Animal Health; culture techniques adopted were essentially those described by Sewell (1961) and Pullan (1968).

The snails were fed on green algae, mainly Oscillatoria spp., growing on the surface of a layer of mud in plastic boxes (Pullan, 1968). Mixing the local soil in a specially formulated mineral solution instead of tap water (Lehner, 1977) and incubating the algal cultures at 23°C under two 30 watt fluorescent strip lights ensured good algal growth.

A small amount of algae from an old green culture transferred to the middle of fresh mud layer covered the surface of the mud after 3-4 days. If not required immediately, the boxes containing the algal cultures were stored in the cold room at 10°C. When so stored, algal cultures became dark green-brown in colour and before use they were rewetted with mineral fluid and placed under strip lights at 23°C overnight to restore their original state.

Some boxes containing adult snails were kept in the cold room for periods of aestivation not exceeding three months. When needed, these were taken out and placed on algal cultures at 23°C.

Egg masses from these snails hatched after about 10 days at 23°C. Strips of algae were put into the boxes of newly hatched snails for the first 2-3 days. The young snails were then carefully transferred by forceps into fresh algal cultures. When they reached a length of 2-5mm the snails were infected with miracidia of F. hepatica.

Eggs of F. hepatica were obtained from the gall bladders of infected sheep from the local abbatoir. They were washed and kept in distilled water in a petri dish at 23°C. The water was changed daily during the first week. Thereafter they were kept in the dark, in an amber bottle covered with aluminium foil at 23°C. On exposure to daylight 2 weeks or more after setting up the culture the miracidia soon hatched and were used as soon as possible to infect the snails.

Each snail was placed individually in a well of a perspex haemagglutination plate containing 5-10 miracidia in a little distilled water. The plates were then covered and left overnight at room temperature. Infected snails were maintained on algal cultures at 23°C and cercariae were shed about 5 weeks after infection.

To induce shedding, the infected snails were put into a small polythene bag (12x7.5cm) half-filled with distilled water at 10°C and left in the light at room temperature. The cercariae were shed as the water warmed up to room temperature. After about 6 hours the snails were removed onto fresh algal cultures at 23°C. Snail debris in the polythene bag containing the metacercariae was gently washed off. The polythene bag was then half filled with distilled water and left at room temperature for a week before transfer to the cold room at 10°C for storage.

3.2. Experimental animals

The rats used in all experiments were Porton Wistar males, 6-7 weeks old at the start of experiments (300-350 g). The rabbits were New Zealand White males about 3 months old (2.5 - 3 Kg). Rats and rabbits were all purchased from the Centre for Laboratory Animals, Bush Estate, Roslin, Midlothian.

Sheep and calves were all males, about 4 months old, Cheviots and Ayrshires respectively. They were obtained from Easter Bush Farm, Roslin, Midlothian.

3.3. Infection of animals

A small piece of polythene bag containing metacercariae was cut off and the metacercariae were examined for viability. Viable metacercariae showed a characteristic granular appearance under the microscope. The required number of viable metacercariae was then scraped off into a watch glass pretreated with silicone "Replecote" (Hopkin and Williams Ltd., Chadwell Heath, Essex).

For the infection of rats, the metacercariae were suspended in 1% gum tragacanth. A pasteur pipette fitted with about 5 cm of 1 mm i.d. plastic tubing and treated with silicone was used to administer metacercariae into the pharynx of a rat which was lightly anaesthetised with ether.

For the infection of rabbits, sheep and calves the required number of viable metacercariae was scraped off the polythene bag and mixed with a small quantity of cellulose powder ('Whatman CF1', Whatman Biochemicals Ltd., Maidstone, Kent) in a watch glass containing few drops of distilled

water. The cellulose powder absorbed the water and the resulting doughy mixture was transferred to a gelatine capsule (Parke Davies and Co., Hounslow, London).

Such a capsule was administered to a rabbit using long forceps to place it at the base of the tongue, while a balling gun was used to infect sheep and calves.

3.4. Faecal examination

Faeceal samples from sheep and calves were examined for F. hepatica eggs to establish maturity of flukes. The Sellotape (Sellotape Products Ltd., Borehamwood, Herts) modification of the zinc sulphate flotation method (Sewell and Hammond, 1972) was used. A suspension of 1 g of faeces in water was centrifuged and the sediment was resuspended in saturated salt solution and again centrifuged. The sediment was then resuspended in zinc sulphate solution of specific gravity 1.3, so that the convex meniscus was slightly above the top of a lipped plastic centrifuge tube. A short length of sellotape was then placed flat over the top of the tube and pressed onto the rim. After centrifugation at 1500 g for 3 minutes the sellotape was examined under the microscope.

In this technique the concentration of the eggs on the sellotape allows the detection of eggs at very low concentrations.

3.5. Collection of blood and serum

3.5.1. Rats

Rats were bled from the tail using the following method: A rubber bung was fixed around the neck of a plastic filter funnel and silicone rubber tubing was used to attach a blood

collecting vial to the neck of the filter funnel. The funnel was then inserted into a conical flask with the vial hanging inside the flask. The funnel was supported tightly to the flask by means of the rubber bung (Plate 3.1.).

After light ether anaesthesia, the tail was swabbed with xylol to engorge the blood vessels and then covered with a thin layer of petroleum jelly to facilitate the flow of blood. A small incision was made towards the tip of the tail leaving sufficient space for succeeding bleedings. The rat was then placed in the filter funnel with the tail extending down the neck of the funnel. About 1 ml of blood was collected into a 2.5 ml plastic vial containing disodium ethylenediamine tetra-acetic acid (EDTA) (Becton, Dickinson, U.K. Ltd., Wembley, Middlesex) as anticoagulant.

For the preparation of serum, blood was collected in vials without an anticoagulant, left overnight at 4°C and centrifuged at 2500 g for 20 minutes. Serum was then collected and kept in plastic vials (Luckham Ltd., Labro Works, Burgess Hill, Sussex) stored at -20°C.

3.5.2. Rabbits

A suitable site was shaved at the margin of the ear and the ear veins were dilated by applying a little xylol. A 20 g needle (Becton, Dickinson, U.K. Ltd., Wembley, Middlesex) was inserted into one of the engorged peripheral veins and about 1 ml of blood was collected into a 2.5 ml plastic vial containing EDTA.

For serum preparation blood was collected in a universal bottle, allowed to clot and kept at 4°C overnight. It was then centrifuged at 2500 g for 20 minutes and the serum was collected

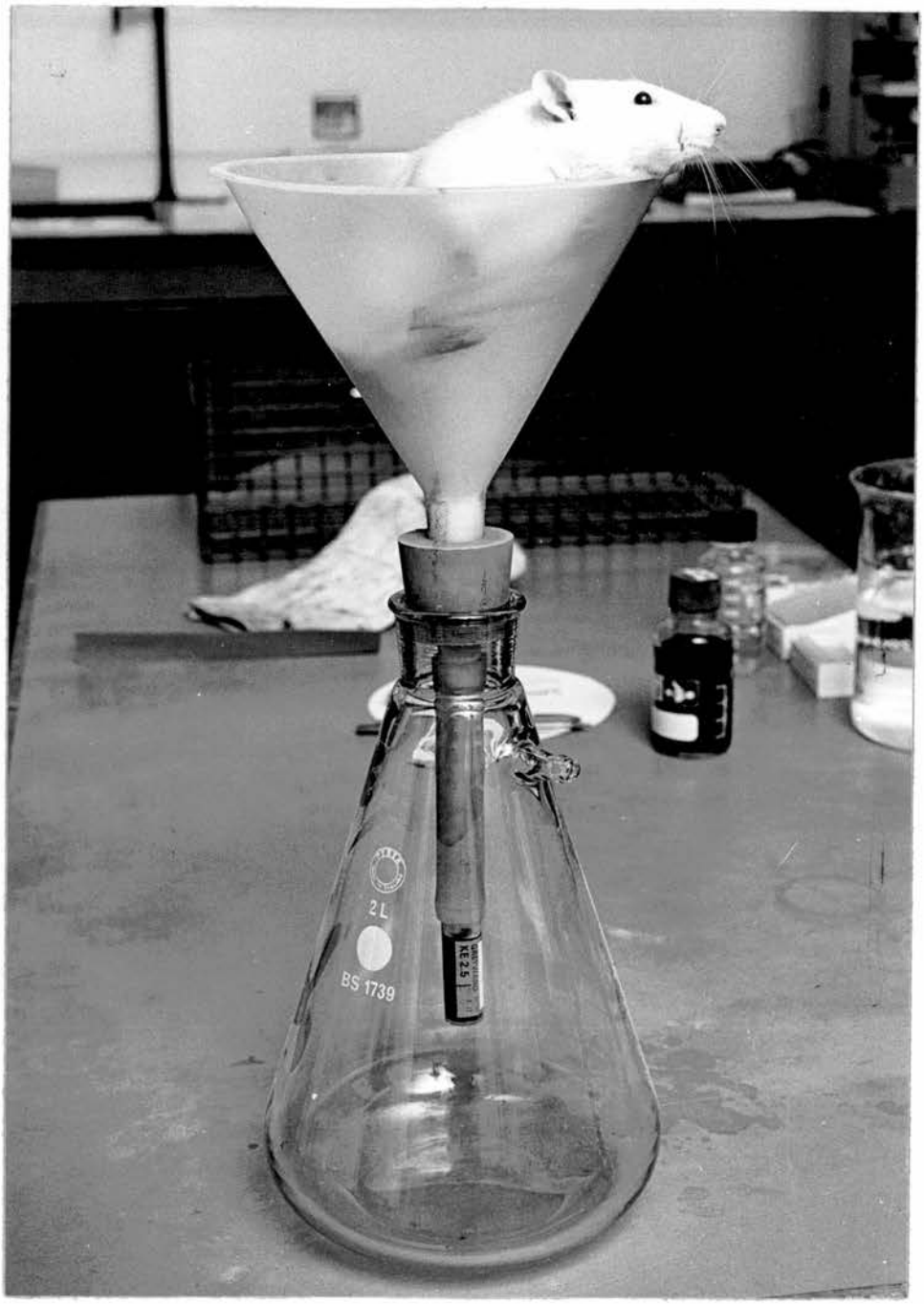


Plate 3.1. Bleeding of a rat.

and kept at -20°C.

3.5.3. Sheep and Calves

Blood was collected into a wide necked plastic jar from the jugular veins of sheep and calves using 18 g disposable hypodermic needles (Sherwood Medical Industries Ltd., Crawley, Sussex) attached to silicone rubber tubing. The blood was left for about an hour at 37°C to clot. To allow better retraction, the clot was then cut into small pieces using a sharp knife and kept at 4°C overnight. The exudate was then centrifuged at 2500 g for 20 minutes and the serum was collected and kept at -20°C.

3.6. Fasciolicidal drugs

Deacetylated diamphenethide (7-bis-2-p-aminophenoxy-ethyl ether) (Wellcome Research Laboratories, Berkhamsted, Herts.) was used at 150 mg/kg body weight to eliminate young and adult F. hepatica in rats. The drug was suspended in a small amount of water and administered, by means of a pasteur pipette with a piece of plastic tubing attached to the end, into the oesophagus of a rat which had been lightly anaesthetised with ether. A pilot experiment (4.1.) proved the efficiency of deacetylated diamphenethide against immature and mature flukes in rats.

Rabbits were treated with rafoxanide "Flukanide" (Merck, Sharp and Dohme Ltd., Hoddesdon, Hertfordshire) at the rate of 26 mg/kg body weight. The dose was administered by stomach tube. Bolbol (1975) had shown rafoxanide to be an effective anthelmintic against the immature stages of F. hepatica in rabbits.

3.7. Post-mortem procedure and recovery of flukes from rats and rabbits

Rats were killed with ether and rabbits were killed by an intravenous injection of Euthatal (Abbot Laboratories, Queenborough, Kent).

The abdominal cavity of each animal was opened, the liver carefully removed and the gross pathological changes recorded.

Adult flukes were recovered by incising the main bile ducts and, in rabbits, the gall bladders. Each liver was sliced into small pieces about 1 cm square, which were kept in saline at 37°C for about 3 hours. Flukes which emerged were removed and the pieces of liver were then gently squeezed to remove any remaining flukes. The total number of flukes recovered from each liver was then recorded.

3.8. Implantation procedures

3.8.1. Source and maintenance of *F. hepatica*

Adult flukes used for implanting into rats were obtained from experimentally infected rats killed with ether 8 weeks after infection. Using sterile instruments, the abdominal cavity of a donor rat was opened in a laminar air-flow cabinet (Microflow Ltd., Fleet, Hants). The liver was removed, the bile duct opened and the flukes transferred into the maintenance medium.

Adult flukes for implanting into rabbits were similarly obtained from experimentally infected rabbits killed by an intravenous injection of pentobarbitone sodium solution

("Euthatal" Abbot Laboratories, Queenborough, Kent) 8 weeks after infection.

Flukes were maintained in sterile 199 tissue culture medium at 37°C until transfer to recepient rats or rabbits.

3.8.2. Implantation of flukes

Pentobarbitone sodium at a rate of 25 mg per kg body weight was used as an anaesthetic. It was given intraperitoneally in rats and intravenously in rabbits.

The right flank of each animal was shaved and cleaned with chlorhexidine (Hibiscrub, Imperial Chemical Industries, Macclesfield, Cheshire) followed by 70% ethyl alchol.

For subcutaneous implantation in rats and rabbits, an incision about 10mm long was made in the skin and the subcutaneous tissue was separated with blunt-ended forceps. The flukes were then carefully inserted into the site and the skin clipped together with 2 or 3 Michel Suture Clips (12 x 2.5 mm) (Thackray Ltd., Leeds, London and Glasgow). The wound was then dressed with sulphanilamide powder (Bayer, U.K. Ltd., Bury St. Edmunds, Suffolk).

Intraperitoneal implantation of flukes was similarly conducted, except that the incised muscle layer was sutured with silk ('Mersilk', Ethicon, Edinburgh) before clipping the skin.

3.8.3. Preparation and implantation of diffusion chambers

Diffusion chambers were constructed under a laminar airflow cabinet. Two membrane filters ('MF Filter', Millipore, U.K. Ltd., London) of pore size 0.45 um were moistened with distilled water and blotted with absorbent paper to remove

excess water. This made the filters swell slightly and allowed them to form a taut cover when dry.

The filters were then glued with 'MF cement' (Millipore, U.K. Ltd., London) to each side of a sterile Plexiglass ring 0.14 mm internal diameter and 2 mm thickness (Millipore, U.K. Ltd., London).

For preparing chambers containing flukes, a moistened filter membrane was glued to one side of the ring. A small quantity of 199 tissue culture medium was then placed in the chamber together with an adult fluke. The second filter membrane was then glued on the top of the ring.

The procedure of subcutaneous or intraperitoneal implantation of diffusion chambers into rats or rabbits was similar to that described for implantation of flukes.

3.8.4. Killing of flukes

Mature flukes were killed before implantation by keeping them at -20°C for 10 minutes.

3.9. Haematological techniques

Blood samples in vials containing EDTA were well mixed in a Matburn Cell Suspension Mixer (Matburn Ltd., London).

For total leucocyte counts an electronic cell counter (Model FN, Coulter Electronics, South Dunstable, Bedfordshire) was used according to the manufacturer's instructions.

Differential leucocyte counts were made using the Undritz modification of the peroxidase staining method (Archer, 1965). This method facilitates the differentiation of the heterophils from the eosinophils, which are difficult to differentiate in

rabbits, using the usual Romanowsky stains (Purvis and Sewell, 1973).

Absolute eosinophil counts were then calculated from the percentage of eosinophils and total leucocyte counts.

3.10. Glutamic dehydrogenase assay (E.C. No. 1.4.1.3)

This was used as an indicator of liver damage (Sewell, 1967a) and carried out by Ford and Boyd's (1962) method. A "SP 1800" spectrophotometer (Pye Unicam, Cambridge) was used to measure the oxidation of diphosphopyridine nucleotide (DPNH) (Sigma Chemical Company Ltd., London) at a wavelength of 340 nm. The reduction in optical density was recorded graphically on an "AR2" Linear Recorder (Pye Unicam, Cambridge). A reduction of optical density of 0.001 is equivalent to the oxidation of 4.83×10^{-4} moles of DPNH and the serum enzyme activity can thus be expressed in micro-moles of product per litre of serum per minute (i.u.s.).

3.11. Buffers

Unless otherwise stated, all buffers were prepared as described by Dawson, Elliott, Elliott and Jones (1969).

3.12. Sterilization Procedures

Surgical instruments were sterilized in a hot air oven at 160°C for 2 hours. Glassware, plexiglass rings used for construction of diffusion chambers and the apparatus used for the continuous-flow culture system were sterilized by autoclaving at 15 lbs. pressure for 20 minutes.

Sodium hydrogen carbonate solution was sterilized by filtration through a 25 mm diam., 0.22 um membrane filter (Millipore U.K. Ltd., London) in a Swinnex filter holder

(Millipore U.K. Ltd., London). Before use the holder and filters were assembled, wrapped in aluminium foil and autoclaved at 15 lbs. pressure for 20 minutes.

3.13. Production of Somatic fluke antigen

Adult flukes collected from the bile ducts of rats or rabbits were washed with physiological saline, chopped into small pieces and homogenised, in about 5 volumes of sodium carbonate buffer (0.1M, pH 9.6), with 0.02% Na N_3, in a universal bottle with a laboratory homogeniser (Silverson Machines Ltd., Waterside, Chesham, Bucks.). The suspension was incubated at 4°C overnight and then centrifuged at 2500 g for 20 minutes. The supernatant was then separated off, its protein content determined and stored at -20°C.

3.14. Production of metabolic antigen

A simplified version of the continuous-flow culture system described by Lehner (1977) was used to produce metabolic antigen from adult F. hepatica.

The culture medium comprised 1 volume of 10x concentrated 199 tissue culture medium (Gibco Bio-Cult, Paisley, Scotland) in 9 volumes of sterile $NaHCO_3$ solution. The $NaHCO_3$ solution was prepared by dissolving 2.2 g of $NaHCO_3$ in a litre of distilled water. Antibiotics added to the culture medium comprised 100 i.u. benzyl penicillin per ml ("Crystapen", Glaxo Laboratories Ltd., Greenford, England), 100 mg per ml streptomycin/dihydrostreptomycin ("Dimycin", Glaxo Laboratories Ltd., Greenford, England) and 2mg per ml amphotericin B ("Fungizone", E.R. Squibb and Sons, Incorporation, New York).

The culture medium was kept in a 2 litre "Pyrex" aspirator (Jobling Laboratory Division, Stone, Staffordshire), which was closed by a silicone rubber bung. The aspirator had a side-outlet at the bottom into which a silicone rubber bung was inserted.

A peristaltic pump (Desaga PLG-multipurpose peristaltic pump 13 2100, Camlab, Cambridge) was used to pump the culture medium from the aspirator through fine bore silicone rubber tubing into a 30cm length of 8mm flat-width nylon sleeving (Portex Ltd., Hythe, Kent), in which the flukes were kept. Each end of the nylon sleeving containing the flukes was tightly fixed to stainless steel tubing by cuffs of silicone rubber tubing, and the steel tubes were supported by clips on a wooden frame.

The used medium containing the metabolic products was then passed through fine bore tubing into a 250 ml conical flask.

All joints in the system were sealed with silicone sealing compound ("Aquaseal", Dow Corning, Windsor) thus preventing leakages.

Two sets of the continuous-flow culture system were assembled and sterilized and 2 litres of culture medium at 37°C were added to each aspirator in a laminar air-flow cabinet (Microflow Ltd., Fleet, Hants). Thirteen adult flukes were placed in the nylon sleeving of one set and 23 adult flukes were placed in the other set.

The two systems were then transferred to the warm room at 37°C and the pump started with two channels, each supplying 16ml/

hour. When it was stopped after 6 days all the flukes were still alive. The medium containing the metabolic products from the two sets was pooled and concentrated using 50% polyethelene glycol 6000 (BDH Chemicals Ltd., Poole, England). It was then dialysed against several changes of phosphate buffered saline (PBS) (pH 7.3) (Oxoid Ltd., London). The protein content of the final product was 4.5 mg/ml.

3.15. Protein Estimation

The technique of Warburg and Christian (1941) was used for the estimation of the protein content in the antigen and gamma-globulin solutions. The extinction of an appropriately diluted protein solution was measured at both 260 nm and 280 nm on "SP 1800" spectrophotometer (Pye Unicam, Cambridge), and the ratio E280/E260 was calculated. Using this ratio, a factor (F) for the calculation of the protein concentration can be read from a standard table (Warburg and Christian, 1941). The protein concentration was calculated by the formula:-

$$\text{Protein concentration (mg/ml)} = \text{Extinction at 280 nm} \times F \times \frac{1}{d}$$

where d = length of light path in cm.

3.16. Serum gamma-globulin precipitation by sodium sulphate

The technique of Stelos (1967) was used to precipitate serum gamma-globulin.

Eighteen grams of sodium sulphate were added per 100 ml of serum at about 25°C. The precipitate was washed twice with 100 ml of 18% sodium sulphate by centrifuging and discarding the supernatant each time. The sediment was dissolved in 40 ml of pH8 borate-buffered saline and the globulin solution again precipitated by adding sodium sulphate to a concentration of

12 g per 100 ml. The precipitate was then washed twice more with 100 ml of 12% sodium sulphate.

The precipitate was then dissolved in 20 ml of pH8 borate buffered saline and the globulin reprecipitated by adding sodium sulphate to a concentration of 12g per 100ml. The precipitated globulins were finally dissolved in about 10 ml of phosphate buffered saline (PBS) pH 7.3 (Oxoid Ltd., London) solution and dialysed against the latter solution to remove the sodium sulphate. The protein content of the sample was then determined and stored at -20°C.

Although a high yield (98%) of gamma-globulin can be obtained by this method (Stelos, 1967), it was found to be inconvenient as the solubility is markedly temperature dependent. Thus precipitation of gamma-globulin with ammonium sulphate was preferred.

3.17. Serum fractionation with ammonium sulphate

Gamma-globulins were precipitated by adding saturated ammonium sulphate to the serum to give a final concentration of one third saturation, as described by Campbell, Garvey, Cremer and Sussdorf (1970).

Saturated ammonium sulphate was prepared by stirring 1000 g ammonium sulphate in a litre of distilled water at 50°C until most of the salt was dissolved. It was then allowed to stand overnight at room temperature. Just prior to use the pH was adjusted to 7.8 using 2N NaOH or sulphuric acid, thus avoiding foreign ions. Saturated ammonium sulphate solution was added dropwise with continual stirring to two volumes of serum, to a final concentration of one-third saturation. The suspension

was stirred by a magnetic stirrer (Gallenkamp and Company Ltd., London) for about 2 hours to avoid mechanical trapping of serum components other than gamma-globulin in the precipitate. Proteins were recovered by centrifugation at 2500 g for 30 minutes. The precipitate was then dissolved in physiological saline to restore the original volume of serum. The gamma-globulin fraction was further purified by a second and third precipitation, after which the precipitate was dissolved in 0.01M phosphate buffer (pH 6.8) to a final volume half that of the original serum sample.

The ammonium sulphate was removed by dialysis against repeated changes of 0.01M phosphate buffer (pH 6.8) for several days at 4°C. The dialysate was checked for the presence of sulphate ions by adding a few drops of 10% barium chloride. If sulphate ions were present a white turbidity or precipitate of barium sulphate formed in which case dialysis was continued. The sulphate-free solution was centrifuged at 4°C for 30 minutes at 1400 g to remove any insoluble material and the protein content of the sample was then determined.

3.18. Immuno-precipitation

This was carried out using the LKB immunodiffusion apparatus (LKB Instruments Ltd., South Croydon, Surrey) according to the method described by Sewell (1966b).

One percent agar in barbiturate buffer was used. To prepare this, 5.75 g diethyl barbituric acid and 3.75 g sodium barbitone were dissolved in about 500 ml hot distilled water and the solution was then made up to 2 litres. Five grams of agar (Oxoid Ltd., London), 0.5 g sodium azide and 50 g sodium

chloride were then dissolved in 500 ml of a solution made of 100 ml buffer and 400 ml distilled water. The mixture was boiled to dissolve the agar and the molten agar dispensed into universal bottles and stored at 4°C to await use.

A semi-quantitative immuno-diffusion technique of (Sewell, 1967b) was used to determine the concentration of metabolic antigen which reacted completely with an equal volume of antibody in the gamma-globulin solution.

Equal volume of several dilutions of gamma-globulin solution and metabolic antigen solution were mixed in the wells of a plastic haemagglutination tray. These mixtures were then dispensed into a row of wells cut in the agar using a modification of the LKB apparatus described by Sewell (1966b). A parallel, but off-set row of wells contained an indicator system, consisting of undiluted gamma-globulin solution and metabolic antigen dispensed into alternate wells.

Any excess antibody or antigen was detected by the deflection of the precipitation line between the upper and lower rows of wells. The equivalence end-point was indicated by an undeflected precipitin line.

3.19. Enzyme-linked immunosorbent assay (ELISA)

The microplate system described for ELISA by Ruitenberg, Steerenberg and Brosi (1975) was used with some modifications. A pilot experiment showed that the use of O-phenylenediamine (O-diaminobenzene) dihydrochloride (OPD) (Sigma Chemical Company Ltd., London) in the substrate instead of 5 amino-salicylic acid (5AS) (Cambrian Chemicals Ltd., Croydon) made the ELISA assays of serum samples from rats less expensive.

The use of 5AS and H_2O_2 as substrate required an enzyme conjugate at a dilution of 1/50 whereas the enzyme conjugate dilution needed for an assay using OPD and H_2O_2 was only 1/400 (Appendix Tables 2.1, 2.2.); therefore OPD and H_2O_2 were used as the substrate for the assay of all serum samples from rats and rabbits.

With the change of substrate it was found preferable to double the periods of incubation in antiserum and enzyme conjugate to 60 minutes. The period of incubation in the substrate however, was halved to 30 minutes because of the more rapid development of the colour.

The assay procedure was as follows:

The wells of Titertek/Linbro polystyrene, flat-bottomed, microtitre trays (Flow Laboratories Ltd., Irvine, Ayrshire) were coated with 0.3 ml somatic antigen solution containing 4.5 ug/ml protein in 0.1M sodium carbonate buffer (pH 9.6) with 0.02% NaN_3 (Appendix Tables 2.3, 2.4.). The trays were then incubated for 3 hours at 37°C and stored at 4°C until use. Before assay, the trays were washed 3 times, for 5 minutes each time, with 0.9% NaCl containing 0.05 % Tween 20 (Polyoxyethylene sorbitan monolaurate, Sigma Chemical Company Ltd., London).

The optimum serum dilution for the assay of serum samples from rats was 1/25 and that for samples from rabbits was 1/50 (Appendix Tables 2.5, 2.6.). Serum samples were diluted with PBS containing 0.05% Tween 20 and 0.02% NaN_3 and 0.3 ml of the diluted serum was added to each well. After incubation for 60 minutes at 37°C, the trays were again washed 3 times,

for 5 minutes each time, with 0.9% NaCl containing 0.05% Tween 20.

The indicator enzyme was EC 1.11.1.7 horseradish peroxidase (HRPO). When testing rat serum, horseradish peroxidase - conjugated rabbit anti-rat IgG (Nordic Immunological Laboratories, Maidenhead, Berks.) was used at a dilution of 1/50 or 1/400 depending on whether the substrate used in the assay contained 5 AS or OPD. In the assay of rabbit serum, horseradish peroxidase - conjugated goat anti-rabbit IgG (Nordic Laboratories, Maidenhead, Berks.) was used at a dilution of 1/500.

After incubation in the enzyme - conjugated anti-immunoglobulin for 60 minutes at 37°C, trays were again washed 3 times for 5 minutes each time with 0.9% NaCl containing 0.05% Tween 20. Then 0.3 ml of substrate was added to each well.

The substrates for horseradish peroxidase - conjugated anti-immunoglobulin were either 5AS and H_2O_2 (Ruitenberg et al., 1975) or OPD and H_2O_2 (Wolters, Kuijpers, Kacaki and Schuurs, 1976). The former substrate was prepared by dissolving 80 mg 5AS in 100ml hot distilled water and adjusting to pH 6.0 with 1M NaOH prior to use. Nine volumes of 5AS solution were then added to one volume of 0.05% H_2O_2 (30% w/v) to make the working substrate. With this substrate the reaction was stopped after an hour by adding 0.025 ml 4M NaOH to each well and the absorbance of the solution was measured at 449 nm on a SP 6 - 200 spectrophotometer (Pye Unicam, Cambridge) using black walled microcells with a 1cm light path.

The OPD substrate was prepared as follows:-

A buffer solution of 0.1M citric acid and 0.2M Na_2HPO_4 (pH 6.0), was made up, 35 mg OPD dissolved in 100 ml of the buffer and 0.167 ml H_2O_2 (30% w/v) added. The trays containing substrate solution were kept in the dark by wrapping the container in aluminium foil. The reaction was stopped after 30 minutes with 0.025 ml of 1M H_2SO_4 (Aristar). The absorbance of the solution was measured at 460 nm on a Pye Unicam spectrophotometer, using black walled microcells with a 1 cm light path.

3.20. Absorption techniques

3.20.1. Absorption of immune serum by direct contact with adult flukes

Using sterile instruments in a laminar air-flow cabinet (Microflow Ltd., Fleet, Hants.) 16 adult flukes were collected from the bile ducts of infected rats and washed in sterile 199 tissue culture medium at 37°C. They were then placed in 160 ml of immune bovine serum which had been sterilized by filtration through 0.22 µm membrane filter (Millipore U.K.) Ltd., London). After 48 hours at 37°C, the flukes were removed and the serum was refiltered as above and stored at -20°C.

3.20.2. Absorption of immune serum by diffusible antigens from encapsulated flukes

Adult flukes were encapsulated in diffusion chambers as previously described. The procedure for absorption of immune serum by direct contact was then repeated using these in place of free flukes.

3.20.3. Absorption of gamma-globulin from immune serum by metabolic products

The metabolic products collected from adult flukes *in vitro* (3.14.) were used to absorb gamma-globulin from immune bovine serum. The concentration of metabolic antigen which reacted completely with an equal volume of antibody in the gamma-globulin was determined by the semi quantitative immuno-diffusion technique (Sewell, 1967b), (3.18.)

CHAPTER 4

STUDIES ON THE STIMULATION OF RESISTANCE TO FASCIOLA HEPATICA IN RATS AND RABBITS FOLLOWING PREVIOUS INFECTION AND TREATMENT

Introduction

In this chapter studies on the effect of previous immature and mature infections, which had been eliminated by an anthelmintic, on the resistance to challenge with F. hepatica in rats and rabbits are described. Rats were chosen to represent hosts that can give good resistance and rabbits to represent those in which the development of resistance is relatively inneffective. Immature and mature sensitising infections were eliminated in rats and rabbits using deacetylated diamphenethide and rafoxanide respectively. The choice of the latter drug was based on the reports of Bolbol (1975) and Coles (1975) that rafoxanide is an effective fasciolicide in rabbits. However, the use of deacetylated diamphenethide necessitated the following preliminary experiment:

4.1. The activity of deacetylated diamphenethide against immature and mature F. hepatica in rats

Introduction

The fasciolicidal activity of diamphenethide against all stages of F. hepatica in sheep has been reported by Dickerson, Harfenist and Kingsbury (1971) and Kingsbury and Rowland (1972). Harfenist (1973) investigated its mode of action and reported that diamphenethide is active by virtue of its deacetylation in the liver to give an amino compound which is

lethal to F. hepatica. However, Coles (1975) found that the acetylated compound is inactive in the rat. He later (1976) reported that this lack of activity in the rat is due to this animal's inability to deacetylate the drug to the active moiety. This experiment was designed to confirm the fasciolicidal activity of deacetylated diamphenethide in rats.

Experimental design

Twelve rats were divided into 3 equal groups A, B and C. They were all infected orally at day 0 with 20 metacercariae of F. hepatica. Each rat in group A was given an oral dose of deacetylated diamphenethide at a rate of 150 mg/kg body weight at the 4th and again at the 5th week after infection. Group B was similarly treated at the 8th and 9th week and group C was left untreated as a control for infection. Peripheral eosinophil counts and serum glutamic dehydrogenase levels were determined fortnightly and post-mortem examination was carried out 10 weeks after infection.

Results

All rats survived until the end of the experiment. The numbers of flukes recovered at post-mortem examination are shown in Table 4.1., the peripheral eosinophil counts in Appendix Table 4.1. and Fig. 4.1. and the serum glutamic dehydrogenase assays in Appendix Table 4.2. and Fig. 4.2. No flukes were recovered from groups A or B whereas some were recovered from all the rats in the control group C.

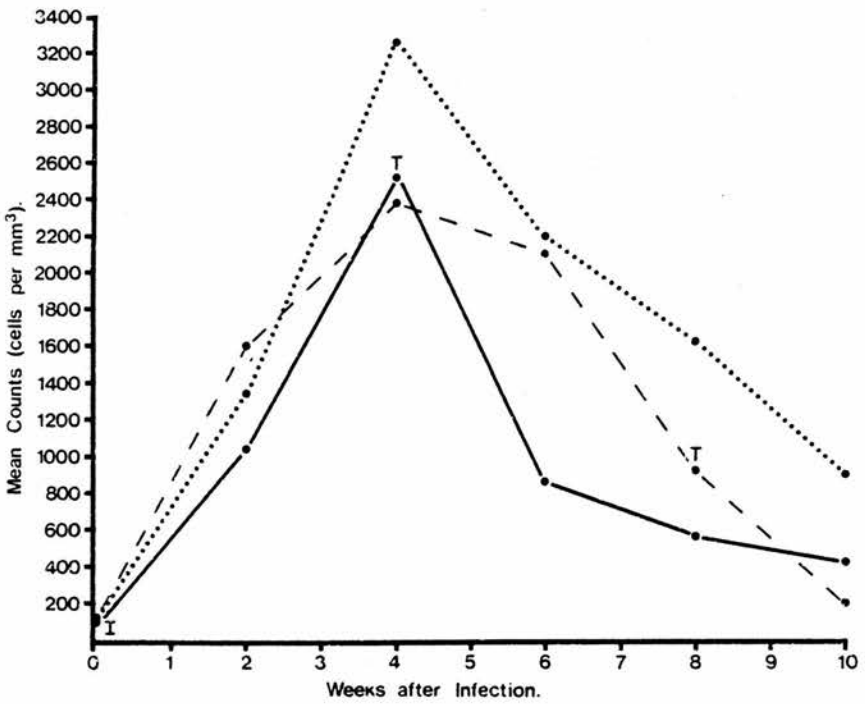
Peripheral eosinophil counts and serum glutamic dehydrogenase levels in group C showed progressive elevation

TABLE 4.1.

Post-mortem findings from rats infected with 20 metacercariae of F. hepatica and later treated with deacetylated diamphenethide

Group	No. of rats	Treatment at (w.p.i.)	Nos. of flukes recovered 10 w.p.i.
A	4	4 and 5	Nil
B	4	8 and 9	Nil
C	4	None	2, 2, 3, 3

Fig.4·1. Peripheral Eosinophil Counts.



Group A ——— Rats infected at week 0 and treated at week 4.

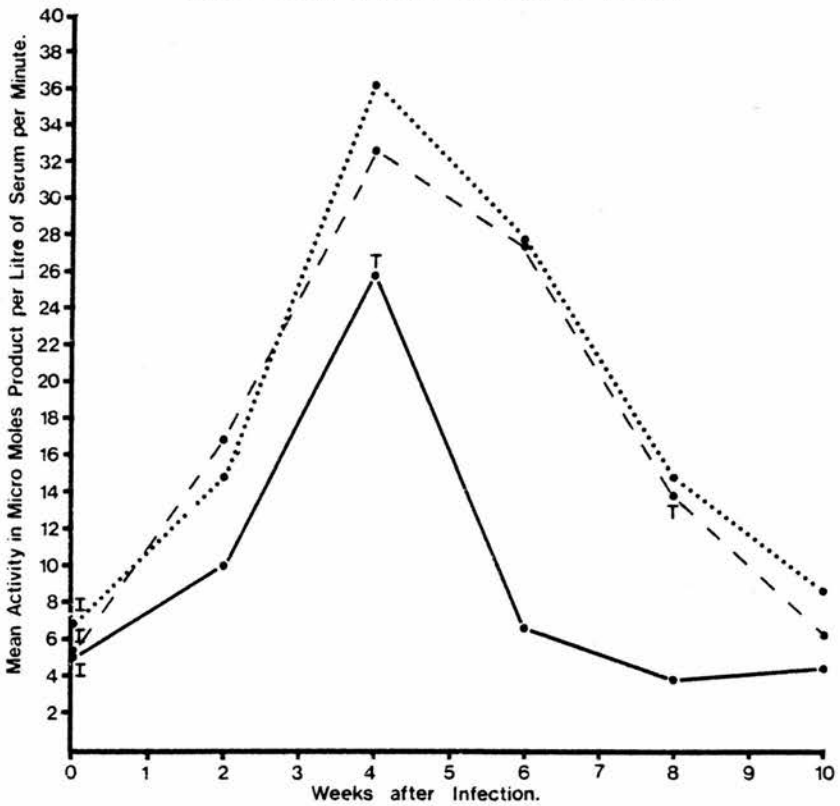
Group B — — Rats infected at week 0 and treated at week 8.

Group C..... Infection controls.

I Infection with 20 metacercariae of F.hepatica.

T Treatment with deacetylated diamphenethide. (150mg/kg).

Fig.4·2. Serum Glutamic Dehydrogenase Assays.



Group A—— Rats infected at week 0 and treated at week 4.

Group B— — Rats infected at week 0 and treated at week 8.

Group C..... Infection controls.

I Infection with 20 metacercariae of F.hepatica

T Treatment with deacetyleted diamphenethide (150 mg/kg).

reaching peaks at the 4th week after infection. The levels then fell steadily. A similar pattern was found in group B which was treated at the 8th week when the levels of those parameters were already fairly low. However, a sharp somewhat premature drop in the levels for both parameters was observed after treatment of the immature infection of group A. The mean enzyme activities of group A were significantly lower than those of group C from the second to the 8th week after infection. (At the 2nd week, $t = 2.55$, $P < 0.05$; at the 4th week, $t = 3.40$, $P < 0.02$; at the 6th week, $t = 14.39$, $P < 0.001$, at the 8th week $t = 4.75$, $P < 0.01$).

Discussion

Coles (1976) found that the free amine of diamphenethide completely eliminated mature F. hepatica in rats but that rat liver extracts failed to deacetylate diamphenethide. The results of the present experiment confirmed that deacetylated diamphenethide was entirely effective against both immature and mature F. hepatica in rats. The peripheral eosinophil counts and serum glutamic dehydrogenase assays were consistent with the post-mortem findings.

4.2. Stimulation of resistance to Fasciola hepatica in rats by two previous immature infections and by a mature infection eliminated in each case by an anthelmintic

Introduction

There is good evidence that rats can acquire the

ability to resist superinfection (Ruther, 1963; Hayes, Bailer and Mitrovic, 1972; 1973; 1974a; Goose and Macgregor, 1973a; b; Doy, Hughes and Harness, 1978) and that elimination of a mature sensitising infection by an anthelmintic does not interfere with this resistance (Goose and Macgregor, 1973a, b; Armour and Dargie, 1974). However, the protective effect of sensitising immature infections, terminated by anthelmintics shortly before challenge has not been investigated in rats. The following experiment is designed to study such resistance and compare it with that stimulated by mature infections terminated by anthelmintics.

Experimental design

Fortytwo rats were divided into the following groups: A (14 rats), B (12 rats), C (8 rats), D_1 (4 rats) and group D_2 (4 rats). Each rat in groups A and D_1 was infected at week 0 with 20 metacercariae of F. hepatica. The rats in group D_1 were the first infection controls and were killed 8 weeks after infection. At the 4th week post-infection each rat in group A was treated with 150 mg/kg body weight of deacetylated diamphenthide. This was repeated at the 5th week, then at the 6th week the rats were reinfected as at week 0. Rats in group D_2 were used as controls for this second infection and were killed 8 weeks later. At week 10 and 11 the rats in group A were treated with deacetylated diamphenethide as before and challenged with 20 metacercariae of F. hepatica at week 12. Post-mortem examinations were carried out 8 weeks later.

Group B received an infective dose equal to that of group A at week 0, similar treatment with deacetylated diamphenthide at weeks 10 and 11 (but not at weeks 4 and 5), challenge at week 12 and necropsy 8 weeks later. Group C was used as a control for the challenge infections for groups A and B.

Peripheral eosinophil counts, serum glutamic dehydrogenase assays and antibody (ELISA) titres were determined every two weeks until the end of the experiment.

Results

Fluke recovery

The mean number of flukes recovered from the first infection controls (group D_1) was 2.5 ± 0.5 and that from the second infection controls (group D_2) was 2.25 ± 1.5.

The fluke burdens recovered from group A, B and C are shown in Table 4.2. Statistical analysis revealed significant reductions of 69% and 83% in groups A and B respectively as compared with group C. However, no significant difference was found between the numbers of flukes recovered from group A as compared to those for group B.

Macroscopic lesions

The macroscopic lesions in the livers of rats challenged after treatment of 2 immature infections (group A, Plates 4.1.1., 4.1.2.) were much more severe than those in the livers of rats challenged after treatment of mature infections (group B, Plates 4.2.1., 4.2.2.) or the livers of the challenge controls (group C, Plates 4.3.1., 4.3.2.). Livers of the rats in group A were generally congested and many

TABLE 4.2.

Stimulation of resistance to F. hepatica in rats by previous infections eliminated by an anthelmintic

Rats group	challenge infection (metacercariae)	No. of flukes recovered at p.m.	
		Individual	mean ± S.D.
Two previous infections and treatments before challenge (A)	20	0, 0, 0, 0, 0, 0, 1, 1, 1, 1, 1, 2 2, 3	0.9 ± 0.9
Challenge following treatment of a mature infection (B)	20	0, 0, 0, 0, 0, 0 0, 0, 0, 1, 2, 3	0.5 ± 1.0
Challenge controls (C)	20	1, 2, 2, 2, 2, 3, 5, 6	2.9 ± 1.7

By Wilcoxon's two-sample test:

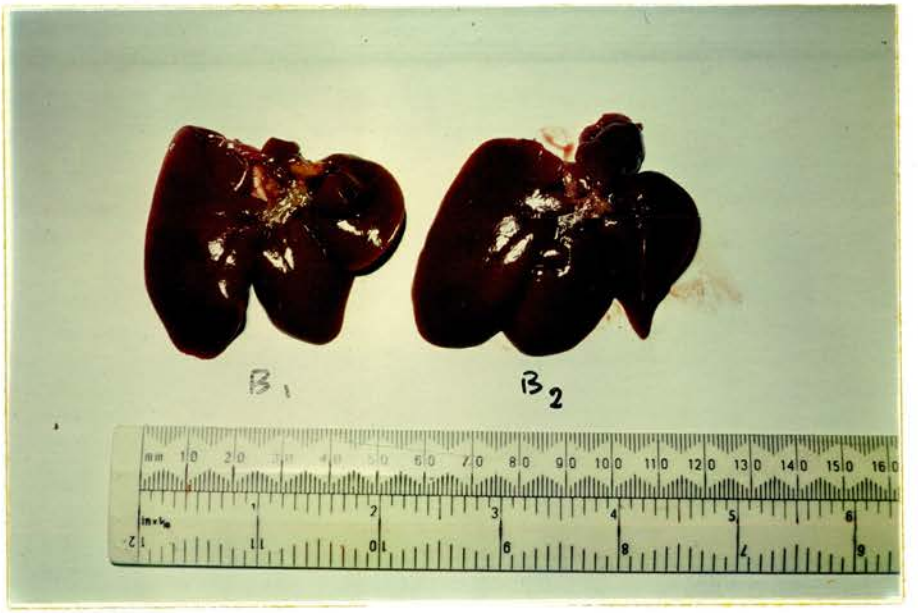
A v B, $P > 0.05$, n.s.

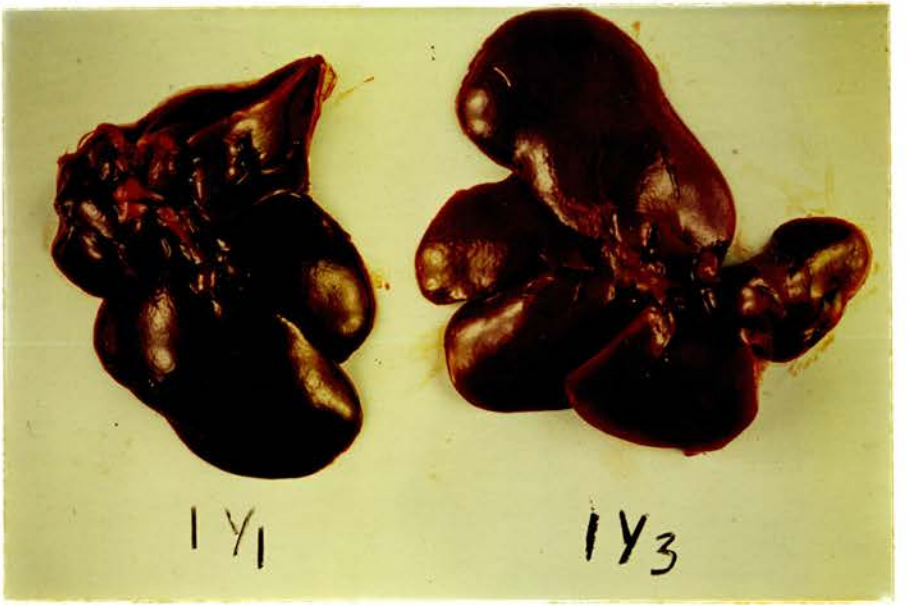
A v C, $P < 0.005$

B v C, $P < 0.001$

Plates 4.1.1., 4.1.2. Macroscopic lesions in the livers of rats challenged with 20 metacercariae of *F. hepatica* after treatment of 2 immature infections.

Plates 4.2.1., 4.2.2. Macroscopic lesions in the livers of rats challenged with 20 metacercariae of F. hepatica after treatment of a mature infection.





Plates 4.3.1., 4.3.2. Macroscopic lesions in the livers of rats challenged with 20 metacercariae of F. hepatica for 8 weeks.

had petechial or ecchymotic haemorrhages. They were hard in consistency and fibrous adhesions were common between adjacent lobes. Diffuse fatty changes as well as yellowish necrotic foci gave many livers a mottled appearance. Necrotic areas were commonly found at the edges of lobes. Some of the necrotic areas protruded from the liver surface in a plaque-like or granular form. Also nodular growth was seen on the visceral and parietal surfaces of many lobes. These nodules were firm in consistency and were about 2-5mm in diameter. The livers in group A were generally smaller than those in group B and C. The nodularity was less marked in most cases in the livers from group B while it was rarely seen in the livers from group C.

The bile ducts containing flukes were generally thickened and dilated. In many of the livers in group C this was the only gross pathological change.

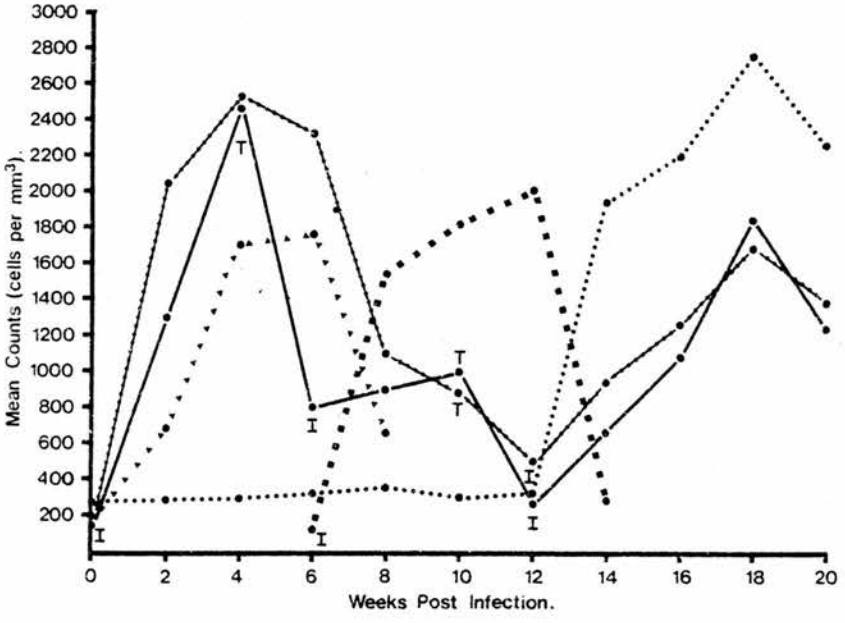
Eosinophil counts

The peripheral eosinophil counts are shown in Appendix Table 4.3. and Fig. 4.3. Infection control groups C, D_1 and D_2 showed progressive increases in eosinophil counts until the 4th or 6th week. The eosinophil counts in groups A and B increased after each infection and decreased after each treatment but reached lower peaks after challenge than after the initial infections.

Serum glutamic dehydrogenase assay

The serum glutamic dehydrogenase levels in the different groups showed similar patterns to those of the eosinophil counts. The data are given in Appendix Table 4.4. and Fig. 4.4. The enzyme levels reached a peak at the 6th week

Fig. 4·3. Peripheral Eosinophil Counts.



Group A ——— Rats infected at weeks 0 and 6, treated at weeks 4 and 10 and challenged at week 12.

Group B – – – Rats infected at week 0, treated at week 10 and challenged at week 12.

Group C····· Rats challenged at week 12.

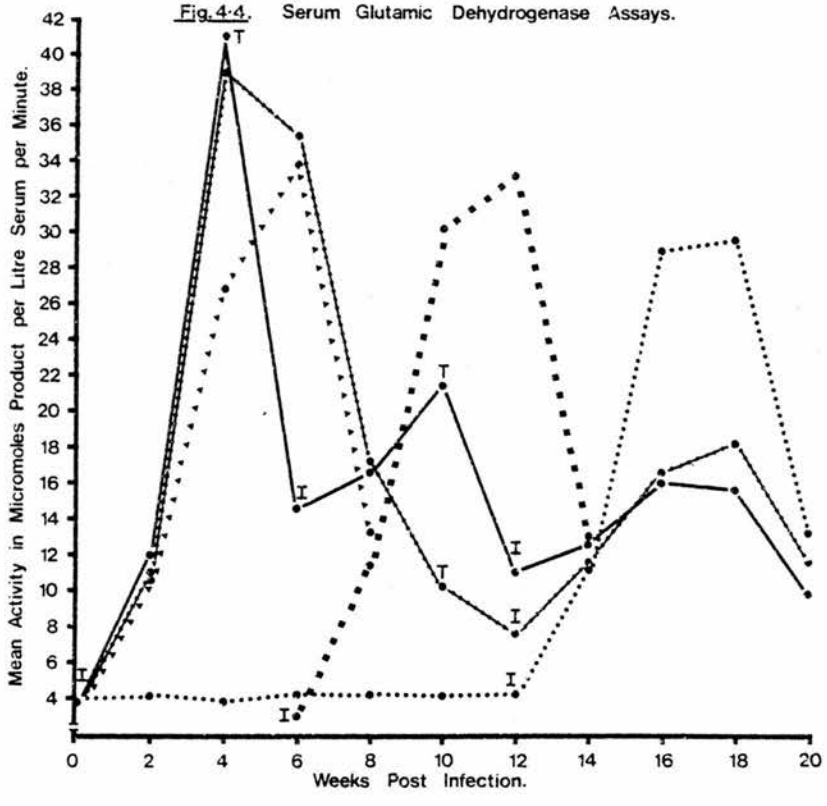
Group D_1 ▲ ▲ Rats used as first infection controls.

Group D_2 ■ ■ Rats used as second infection controls.

I Infection with 20 metacercariae of F.hepatica.

T Treatment with deacetylated diamphenethide. (150mg/kg).

Fig. 4·4. Serum Glutamic Dehydrogenase Assays.



Group A ——— Rats infected at weeks 0 and 6, treated at weeks 4 and 10 and challenged at week 12.

Group B — — Rats infected at week 0, treated at week 10 and challenged at week 12.

Group C .... Rats infected at week 12.

Group D_1 ▲▲▲ Rats used as first infection controls.

Group D_2 ■■■ Rats used as second infection controls.

I Infection with 100 metacercariae of F. hepatica.

T Treatment with deacetylated diamphenethide. (150mg/kg).

post-infection in all three infection control groups. The enzyme levels in groups A and B were consistently elevated after infections and reduced after treatments. The mean enzyme levels in groups A and B were significantly lower than those of the challenge control (group C) at the 4th and 6th weeks after challenge i.e. 16 and 18 weeks after the start of the experiment (A v C at the 16th week, $t = 4.2$, $P < 0.001$; at the 18th week, $t = 5.71$, $P < 0.001$; B v C at the 16th week, $t = 3.93$, $P < 0.001$; at the 18th week, $t = 5.77$, $P < 0.001$).

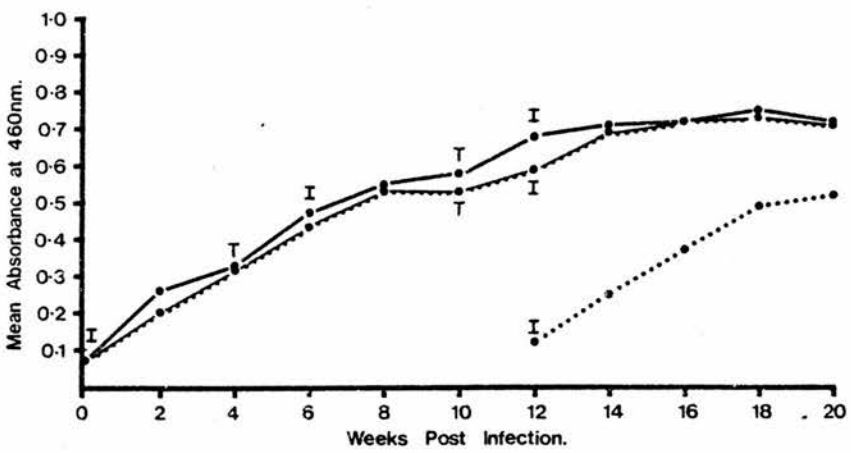
Serology

ELISA values are shown in Appendix Table 4.5. and Fig. 4.5. Those for the control group C showed a progressive elevation after infection up to the 8th week. The antibody titres in groups A and B increased steadily after infection despite the two treatments, to reach a plateau 14 weeks after initial infection.

Discussion

The results show clearly that either immature or mature infections which have been eliminated by anthelmintics can stimulate resistance to challenge in rats. This resistance was not only shown by a significant reduction in the number of flukes recovered from previously infected and treated rats but also by the less marked host response to challenge infection as indicated by lower eosinophil counts and lower glutamic dehydrogenase levels. The level of resistance

Fig. 4·5. ELISA Values.



Group A —— Rats infected · weeks 0 and 6, treated · weeks 4 and 10 and challenged at week 12.

Group B - - - - Rats infected · week 0, treated at week 10 and challenged at week 12.

Group C····· Rats challenge at week 12.

I Infection with 20 metacercaria of F. hepatica.

T Treatment with deacetylated diamphen thide. (150mg/kg).

stimulated by the mature infection, as manifested by the number of flukes recovered at necropsy may have been higher than that initiated by two previous immature infections and treatments but this difference was not significant.

Peripheral eosinophil counts

Although there was a consistently greater eosinophilic response to primary infection than to subsequent infections there was always some increase. Such an eosinophilia is a characteristic feature of parasitic infections and Doy, Hughes and Harness (1978) reported that eosinophils were prevalent in the lamina propia of the small intestine of rats 3 weeks after primary infection and increased markedly 48 hours after challenge.

Serum glutamic dehydrogenase

Boyd (1962) observed a relationship between the increase in serum glutamic dehydrogenase level and the extent of hepatic necrosis due to carbon tetrachloride in sheep, cattle and rats. However, he found that the increase in the serum enzyme activity in the rat was comparatively lower than in sheep and cattle despite the higher concentration of the enzyme in the livers of rats. Accordingly, the author suggested that a most sensitive indicator of hepatic necrosis in the rat would be glutamic pyruvic transaminase (EC 2.6.1.2 Alanine aminotransferase).

The suitability of serum glutamic dehydrogenase as an indicator of hepatic damage to fascioliasis was reported in sheep by Sewell (1967a). This was confirmed by Hughes, Treacher and Harness (1973) in goats and by Lehner (1977) in

rats. The former authors attributed the surprising results of Boyd (1962) to the fact that he used carbon tetrachloride to produce the hepatic damage.

The present findings support those of Lehner (1977) and also show the effect of repeated infections and treatments prior to challenge on the serum levels of this enzyme in the rat. They indicate that the enzyme levels peaked at the 6th week after infection. This is in agreement with the findings of Thorpe (1965) that the maximum liver damage is reached 6 weeks after infection in rats and that from then on flukes enter the bile ducts and further marked hepatic damage is unlikely to occur. Individual variation in the period of hepatic migration may explain the difference in the time of occurrance of maximum hepatic damage in different rats.

Serum glutamic dehydrogenase levels recorded after secondary infections were markedly lower than those following initial infection, indicating less hepatic damage.

Campbell and Barry (1970) reported that serum glutamic-oxalo-acetic transaminase (EC 2.6.1.1 Aspartate amino-transferase) levels in rats decreased after treatment and suggested the use of assays for this enzyme in the selection of compounds effective against liver flukes. However, it would seem that serum glutamic dehydrogenase may be a better alternative for this purpose since it is a liver specific enzyme.

Serology

The enzyme-linked immunosorbent assay (ELISA) of

Engvall and Perlmann (1971) was first used in the serodiagnosis of Trichenella spiralis by Ruitenberg, Steerenberg, Brosi, Buys, Ljungstrom and Engvall (1974). The use of this test in serological studies of many other parasitic diseases has been reviewed by Voller, Bartlett and Bidwell (1976). However, ELISA has only recently been used for the assay of antibodies to F. hepatica by Lehner (1977) in rats, rabbits, sheep and cattle sera and by Burden and Hammet (1978) in bovine serum.

Lehner (1977) reported that metabolic products derived from adult flukes in vitro did not protect rats from challenge with F. hepatica. Booster vaccine injections in such vaccinated rats failed to produce an anamnastic response. He found that after challenge the antibody titres of the vaccinated groups did not rise in proportion to those of the control groups. Sometimes the rise in the titres of the vaccinated rats was even less than that of the controls. However, the present results indicate that the rats which acquired resistance to challenge by curtailed previous immature or mature infections had a different serological response, as measured by ELISA, from that of Lehner's rats vaccinated with metabolic products. The ELISA values continued to rise until 14 - 18 weeks after initial infection in all the groups.

4.3. Stimulation of resistance to Fasciola hepatica in rabbits by two previous infections eliminated by an anthelmintic

Introduction

Bolbol (1975) reported that while a single previous in-

fection curtailed by treatment failed to stimulate resistance to F. hepatica in rabbits, 2 previous infections and treatments resulted in a significant reduction in the number of flukes recovered after challenge. The following experiment was designed to confirm these findings.

Experimental design

Seventeen rabbits were divided into 4 groups, A (6 rabbits), B (7 rabbits), C (2 rabbits) and D (2 rabbits). Each rabbit in groups A and C was infected with 100 metacercariae of F. hepatica at day 0. The rabbits in group A were then treated with rafoxanide at 26 mg/kg body weight at the 4th and again at the 5th week after infection. At the 6th week the rabbits in this group and those in group D were infected with 100 metacercariae. The rabbits in group A were again treated with rafoxanide at the 10th and 11th week, and challenged, together with the rabbits in group B, with 100 metacercariae at the 12th week. The latter group formed the control for the challenge infection. Peripheral eosinophil counts, serum glutamic dehydrogenase levels and antibody titres were determined fortnightly. Post-mortem examination of all rabbits was carried out 8 weeks after their final infection.

Results

Fluke recovery

The numbers of flukes recovered from the previously infected rabbits (group A) and challenge controls (group B) are recorded in Table 4.3. There was a reduction of 33% in the number of flukes recovered from group A compared to group B but this difference was not significant. The mean number of flukes recovered from groups C and D were 30 ± 2 and 28 ± 8 respectively.

TABLE 4.3.

Stimulation of resistance to F. hepatica in rabbits by two previous infections and treatments.

Rabbits group	challenge infected (metacercariae)	Nos. of flukes recovered at PM	
		Individual	mean ± s.d.
Two previous infections and treatments before challenge (A)	100	8, 10, 10, 16, 17, 26	14.5 ± 6.7
Challenge controls (B)	100	15, 16, 16, 21 22, 29, 33	21.7 ± 7.0

By Wilcoxon's two-sample test:

A v B, $P > 0.05$, n.s.

Morbid Anatomy

The gross pathological changes found in the livers of the three infection control groups are shown in plates 4.4., 4.5. and 4.6. They included congestion, enlargement, fatty changes, fibrinous adhesions on the surfaces of all lobes and yellowish or brownish debri in the dilated and thickened bile ducts. Whitish necrotic foci and strands of fibrous connective tissue gave the livers a characteristic appearance. The pathological changes in the livers of the repeatedly infected rabbits (group A) were more severe (Plates 4.7.1., 4.7.2., 4.7.3., 4.7.4.). There were more adhesions and some of the livers were hard to cut due to fibrosis. Granulomas about 1 cm in diameter containing remnants of flukes as well as greenish caseated material protruded from the surfaces of many lobes. The livers in this group had a fleshy appearance.

Peripheral eosinophil counts

The peripheral eosinophil counts are shown in Appendix Table 4.6. and Fig. 4.6. These were clearly influenced by infection and treatment. The mean eosinophil counts of group A progressively increased after infection up to the 4th week when the first treatment was administered. They then dropped slightly but increased again after the second infection. A steep decrease after the second treatment was followed by a steady increase after challenge infection. The challenge infection controls showed progressive increase in mean eosinophil counts until they were killed after 8 weeks. The eosinophil counts of the first and second infection

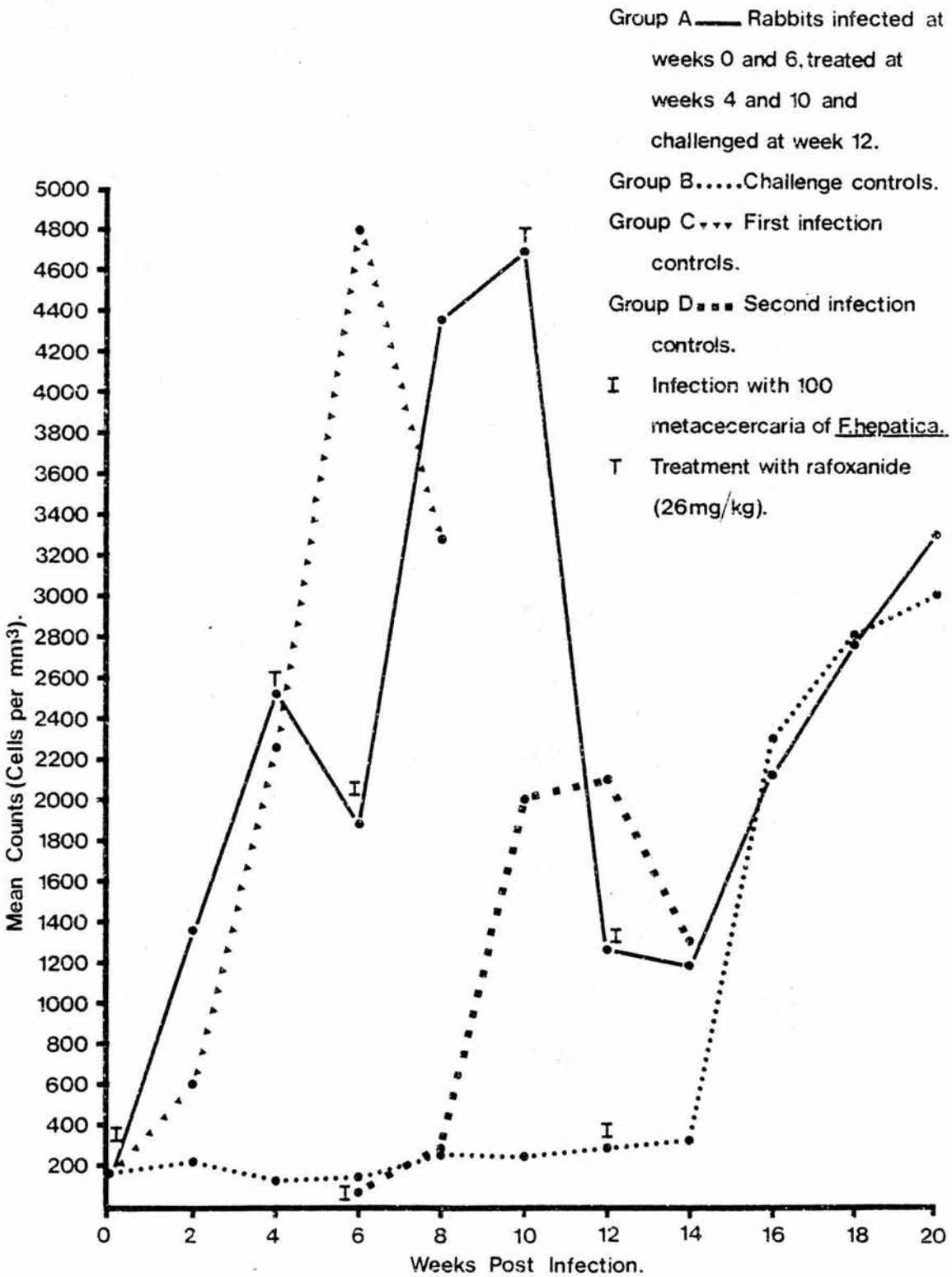
Plates 4.4. and 4.5. Macroscopic lesions in the livers of rabbits challenged with 100 metacercariae of F. hepatica for 8 weeks.

Plate 4.6. Macroscopic lesions in the livers of rabbits challenged with 100 metacercariae of F. hepatica for 8 weeks.

Plates 4.7.1., 4.7.2. Macroscopic lesions in the livers of rabbits challenged with 100 metacercariae of F. hepatica for 8 weeks after treatment of 2 immature previous infections.

Plates 4.7.3., 4.7.4. Macroscopic lesions in the livers of rabbits challenged with 100 metacercariae of F. hepatica for 8 weeks after treatment of 2 immature previous infections.

Fig.4·6. Peripheral Eosinophil Counts.



controls both showed a progressive increase after infection reaching a peak at the 6th week with a decrease afterwards.

Serum-glutamic dehydrogenase assay

The enzyme assays are recorded in Appendix Table 4.7. and Fig. 4.7. Mean levels in all the infection controls (groups B, C and D) showed progressive elevation up to the 6th week followed by a fall at the 8th week. Also, the mean enzyme levels in the repeatedly infected rabbits (group A) rose up to the 4th week, with a slight drop 2 weeks after the first treatment. Mean levels rose again after the second infection, followed by a considerable decrease 2 weeks after the second treatment. A much smaller rise was observed after challenge infection, peaking at the 6th week with a drop in the 8th week. The mean levels in group A were significantly lower at the 4th week ($t = 5.00$, $P < 0.001$) and the 6th week ($t = 5.50$, $P < 0.001$) after challenge infection than those in the challenge infection controls (group B).

Serology

ELISA values are shown in Appendix Table 4.8. and Fig. 4.8. The mean values of the first infection controls rose rapidly up to the 4th week and then remained constant until the 8th week. However, the mean ELISA values of the second infection controls and challenge controls showed a slow but progressive increase until the 8th week after infection. The mean ELISA values of group A rose steadily despite treatment and reinfection to reach a fairly constant plateau by the 10th week, although the highest activity reached was observed in the last week, 20 weeks after initial infection.

Fig.4·7. Serum Glutamic Dehydrogenase Assays.

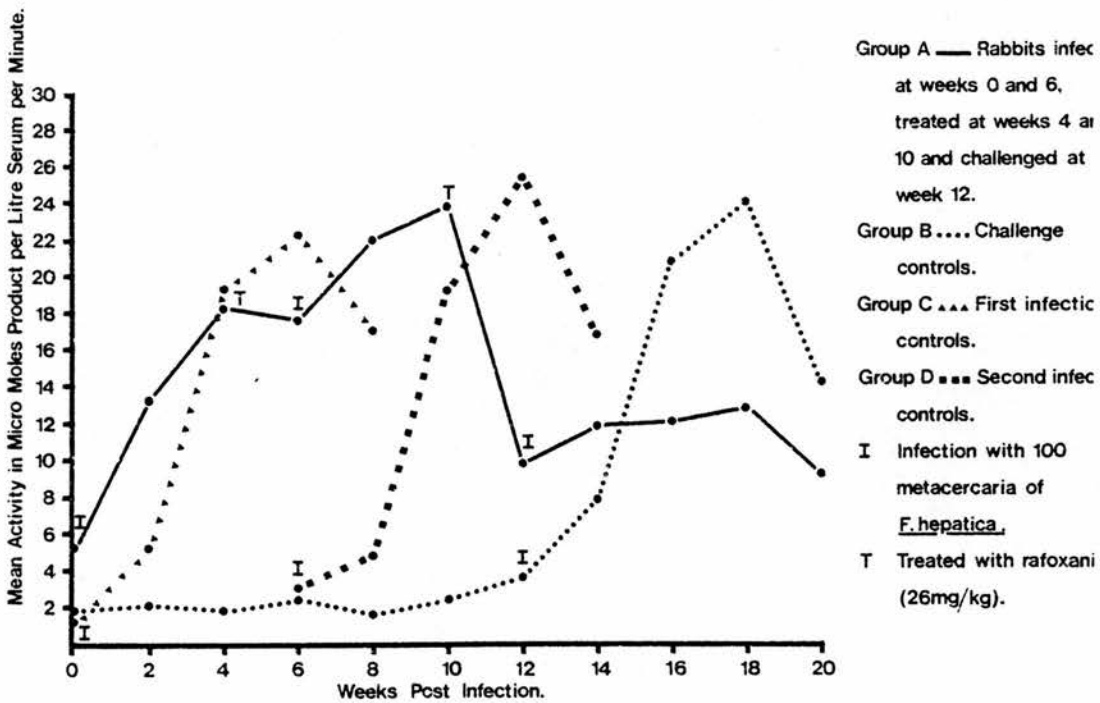
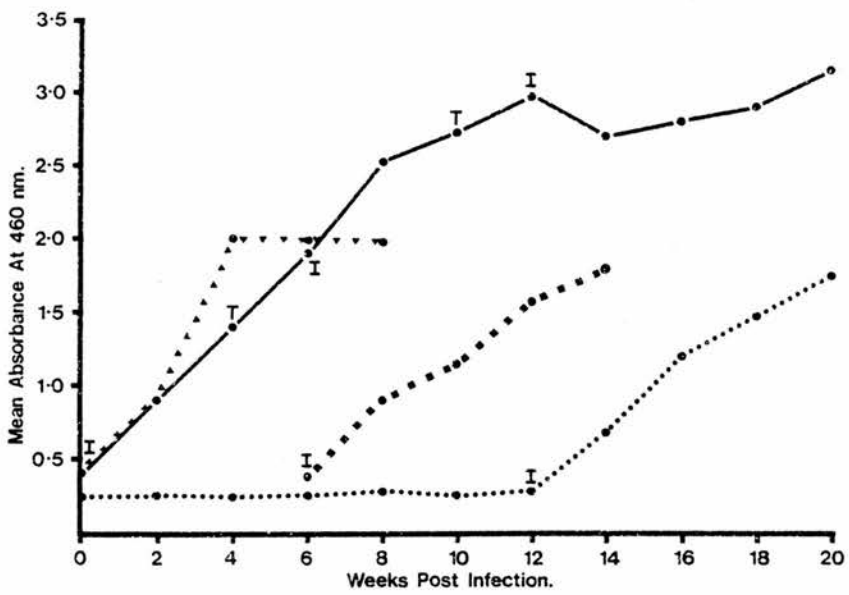


Fig.4·8. ELISA Values.



Group A ——— Rabbits infected at weeks 0 and 6, treated at weeks 4 and 10 and challenged at week 12.

Group B.....Challenged at week 12.

Group C ▾▾▾ First infection controls.

Group D ■■■ Second infection controls.

I Infection with 100 metacercaria of F.hepatica.

T Treatment with rafoxanide (26mg/kg).

Discussion

The results of this experiment will be discussed with those of the following experiment.

4.4. Resistance to Fasciola hepatica in rabbits after treatment of a mature infection

Introduction

In the previous experiments it was found that whereas two sensitising infections which had been treated while the flukes were still immature in the parenchyma failed to stimulate a significant resistance to challenge in rabbits, a similar procedure in rats elicited significant resistance. A slightly higher (though insignificant) level of resistance appeared to have been found in rats when the sensitising infection was removed after maturity. An experiment was therefore designed to find out whether the removal of a mature sensitising infection has a similar effect in rabbits.

Experimental design

Sixteen rabbits were divided into 3 groups, A, B and C. Each of the 6 rabbits in group A was infected with 100 metacercariae of F. hepatica at week 0 together with the two rabbits in group B. These latter were the infection controls for group A. Eight weeks later group A was treated with rafoxanide at 26 mg/kg body weight while group B was necropsied. At week 10, the rabbits in group A were challenged with 100 metacercariae each together with the 8 rabbits of group C. Necropsy was carried out 8 weeks after challenge. Peripheral eosinophil counts and serum glutamic

dehydrogenase assays were determined fortnightly following the challenge infection.

Results

Fluke recovery

The mean number of flukes recovered from the first infection controls (group C) was 44 $\pm$ 2.8. The numbers of flukes recovered from groups A and B are recorded in table 4.4. There was no significant difference between the numbers of flukes recovered from these groups.

Macroscopic lesions

The gross lesions were similar in the livers of rabbits in both groups A and B. These are shown in Plates 4.8.1., 4.8.2. and Plates 4.9.1., 4.9.2., and were similar to those seen in the rabbits which had received single control infections in the previous experiment. Adhesions were found over the surface of the hepatic lobes. Fatty changes, necrotic areas and fibrous networks were characteristic. Large granulomas covered with adhesions were also observed. They had yellowish thick fibrous capsules, containing greenish debri and sometimes yellowish necrotic material resembling solid fat as well as fluke remnants. The bile ducts were enlarged and their walls were thickened.

Peripheral eosinophil counts

These are shown in Appendix Table 4.9. and Fig. 4.9. Eosinophil counts of groups A and B rose steadily to reach a peak at the 6th week after challenge. There was no clear difference between the two groups.

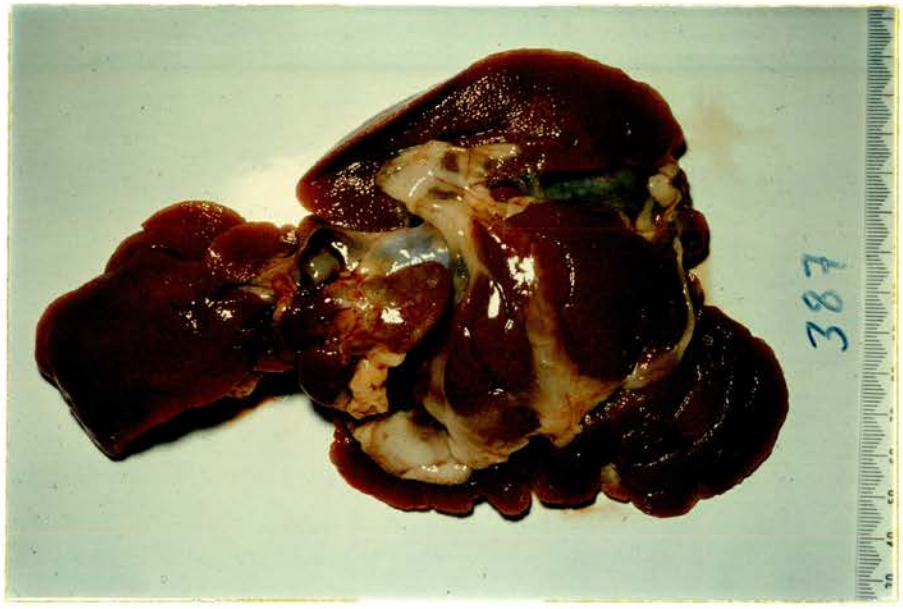
TABLE 4.4.

Resistance to challenge with **F.** hepatica in rabbits following treatment of a mature infection.

Rabbits group	challenge infection (metacercariae)	Nos. of flukes recovered at PM	
		Individual	mean $\pm$ s.d.
Challenge following treatment of a mature infection (A)	100	33, 37, 42 43, 46, 47	41.3 $\pm$ 5.4
Challenge controls (B)	100	32, 32, 36, 41, 44, 49, 53, 58	43.1 $\pm$ 9.7

By Wilcoxon's two-sample test:

A v B, $P > 0.05$, n.s.



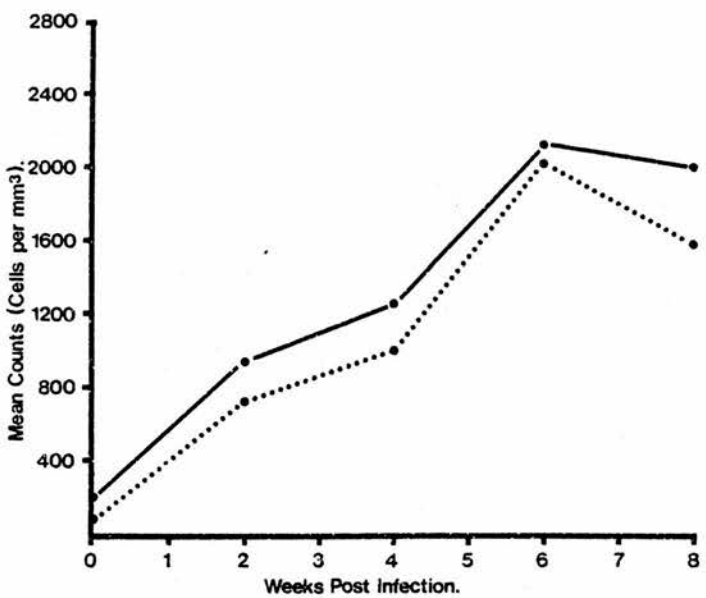
Plates 4.8.1., 4.8.2. Macroscopic lesions in the livers of rabbits challenged with 100 metacercariae of F. hepatica for 8 weeks after treatment of a mature infection.

Plates 4.9.1., 4.9.2. Macroscopic lesions in the livers of rabbits challenged with 100 metacercariae of F. hepatica for 8 weeks.

Fig. 4·9. Peripheral Eosinophil Counts.

Group A ——— Rabbits challeged at week 0 after treatment of an 8-week primary infection.

Group B..... Challenge controls.



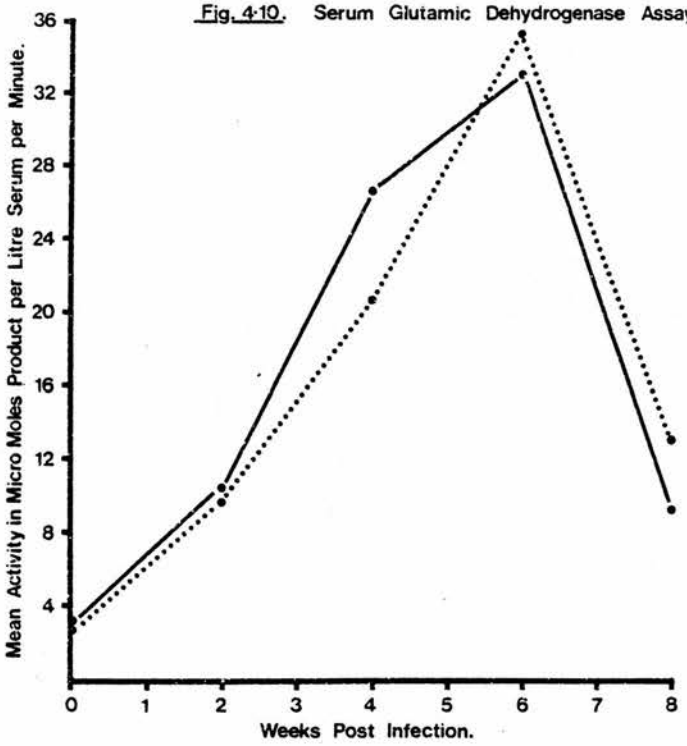
Serum glutamic dehydrogenase

The results of the enzyme assays are shown in Appendix Table 4.10. and Fig. 4.10. Again the levels of the enzyme in both groups A and B rose similarly and steadily to a peak at week 6. There was no significant difference between the mean enzyme levels in the two groups at any time.

Discussion

Contrary to the findings in rats, neither two immature nor one mature infection which had been eliminated by anthelmintics were capable of stimulating a significant resistance to challenge in rabbits. However, the two previous immature infections did result in a reduction of 33% in the challenge infection. Taken together with Bolbol's (1975) findings, this supports his suggestion that such a reduction may be due directly to the structural changes including the fibrosis caused by the repeated immature infections. It is probable that such a reduction did not occur in the rabbits challenged after treatment of a single mature infection because of the lesser degree of damage produced by the latter. Bolbol (1975) also found that a single immature infection did not cause a reduction in the number of flukes developing after challenge. It is also possible that the hepatic parenchymal regeneration after the localisation of the flukes in the bile ducts will have further reduced any impediment to the development of the invading flukes of the challenge infection.

Fig. 4·10. Serum Glutamic Dehydrogenase Assays.



Group A—— Rabbits challenged at week 0 after treatment of an 8-week primary infection.

Group B..... Challenge controls.

Peripheral eosinophil counts

Furmaga, Gundlach and Sobieszewski (1975) reported that the leucocytic changes in sheep and rabbits infected with Fasciola are similar and that the quantitative changes in eosinophils during the different stages of the infection are similar to those in the total leucocyte counts. They reported that a marked eosinophilia occurs between the third and the tenth weeks of infection but this is followed by a fall. The present experiment confirms these findings in rabbits as well as similar findings by Bolbol (1975). The observation that eosinophil counts are readily influenced by treatment has also been reported by Sinclair (1973) in sheep and by Bolbol (1975) in rabbits. However, previous immature or mature infections in rabbits did not appear to influence the eosinophil response to challenge infection; the response in the repeatedly infected animals and infection controls being similar.

Serum glutamic dehydrogenase

The suitability of serum glutamic dehydrogenase as a measure of liver damage in rabbits was not only indicated by the progressive elevation of the enzyme levels during the parenchymal migration phase but also by the influence of the level of infection. Even the insignificant reduction in the number of flukes which developed from the challenge infection in rabbits with 2 previous infections was accompanied by a concomitant decrease in the enzyme level.

Serum glutamic dehydrogenase levels generally peaked 6 weeks after infection. This probably coincides with the period of maximum liver damage which is followed by rapid repair after the localisation of the flukes in the bile ducts. Such observations were also reported by Sewell (1966a).

Serology

Although the ELISA values were higher after challenge in the repeatedly infected rabbits than in the controls, the level of resistance obtained was not significant. Hence, these antibodies do not appear to be protective. Sinclair and Kendall (1969) also showed that while rabbits are capable of producing precipitating antibodies in high concentrations, these were also not protective.

It was perhaps surprising that ELISA values continued to increase after treatment. However, a similar observation has been made by Hillyer and Diaz (1976) studying precipitins. They reported that in rabbits infected with F. hepatica and treated with rafoxanide a further slight rise in precipitin titres occurred but this was followed by a rapid fall. Also Bénex, Guilhon and Barnabé (1973) reported similar findings using a fluorescent antibody technique in sheep treated with bithionol sulphoxide.

CHAPTER 5

STUDIES ON RESISTANCE TO *FASCIOLA HEPATICA* IN RATS AND RABBITS INDUCED BY IMPLANTED FLUKES

The experiments described in chapter 4 indicated that the non-specific hepatic changes caused by previous infections may not be the only factors responsible for the resistance to challenge with *F. hepatica* in rats, although this may be true in rabbits. The following experiments were carried out to confirm this and to show that specific factors derived from the flukes are involved in this resistance.

5.1. The immune response of rats to subcutaneous implantation with mature *F. hepatica*

Introduction

In this experiment mature flukes were implanted subcutaneously into rats to see whether resistance to oral challenge can be stimulated in the absence of the hepatic inflammation and fibrosis which follow primary oral infections.

Experimental design

Twenty rats were divided into 2 groups, A (12 rats) and B (8 rats). Rats in group A were each implanted subcutaneously with 1 or 2 mature flukes. Two weeks later each rat in both groups was orally infected with 20 metacercariae of *F. hepatica*. Peripheral eosinophil counts and serum glutamic dehydrogenase activities were determined fortnightly. Post-mortem examination was carried out 8 weeks after challenge.

Results

At post-mortem examination all the implanted flukes were found to be dead within a fibrous cyst.

Fluke recovery

This is shown in Table 5.1. A highly significant reduction was found in the numbers of flukes recovered from the implanted rats compared with those from the non-implanted controls.

Peripheral eosinophil counts

These are shown in Appendix Table 5.1. and Fig. 5.1. The counts increased after infection in both groups, reaching a peak in the non-implanted rats at the 6th week. In the implanted rats a lower peak was reached 4 weeks after infection.

Serum glutamic dehydrogenase assay

The results are shown in Appendix Table 5.2. and Fig. 5.2. In both groups the enzyme level increased, reaching a peak at the 4th week. However, the increase was much reduced in the implanted rats and the difference between the mean enzyme levels in the two groups was significant at the 4th ($t = 2.70$, $P < 0.02$) and 6th ($t = 3.14$, $P < 0.01$) weeks.

Discussion

The results of this experiment will be discussed together with those of the next experiment.

5.2. The immune response of rabbits to subcutaneous implantation with mature F. hepatica.

Experimental design

Thirteen rabbits were divided into 2 groups, A (8 rabbits) and B (5 rabbits). Rabbits in group A were each implanted subcutaneously with 2, 3 or 5 mature flukes and were challenged 2 weeks later, together with those in group B, with 100 metacercariae each of F. hepatica. Peripheral eosinophil counts

and serum glutamic dehydrogenase activities were determined fortnightly. All rabbits were necropsied 8 weeks after challenge.

Results

At necropsy there were no cysts, similar to those found in rats, around the dead implanted flukes.

Fluke recovery

This is shown in Table 5.2. There was no significant difference in the numbers of flukes recovered from the implanted and non-implanted rabbits.

Peripheral eosinophil counts

These are recorded in Appendix Table 5.3. and Fig. 5.3. Infection was followed by a progressive increase of eosinophil counts reaching a peak at the 6th week in both groups with only slightly higher counts in the non-implanted group, except at six weeks after infection. Even then the difference was not significant.

Serum glutamic dehydrogenase assays

These are shown in Appendix Table 5.4. and Fig. 5.4. Infection was followed in both groups by a progressive elevation of the enzyme level with maximum values at the 6th week. There was no significant difference in the mean enzyme levels in the two groups.

Discussion

Subcutaneous implantation of adult flukes stimulated a significant resistance to oral challenge in rats. This was shown by a significant reduction in the fluke burden and lower glutamic dehydrogenase levels indicating little liver damage. On the other hand, in rabbits there was no evidence of resistance.

TABLE 5.1.

The immune response of rats to subcutaneous implantation with mature F. hepatica.

Rats group	challenge infection (metacercariae)	Nos. of flukes recovered at PM	
		Individual	mean ± s.d.
Implanted with 1 or 2 mature flukes s/c (A)	20	0, 0, 0, 0, 0 0, 0, 0, 1, 1 1, 1	0.3 ± 0.5
Non-implanted (B)	20	1, 1, 2, 2 3, 4, 4, 5	2.8 ± 1.5

By Wilcoxon's two-sample test:

A v B. $P < 0.001$

TABLE 5.2.

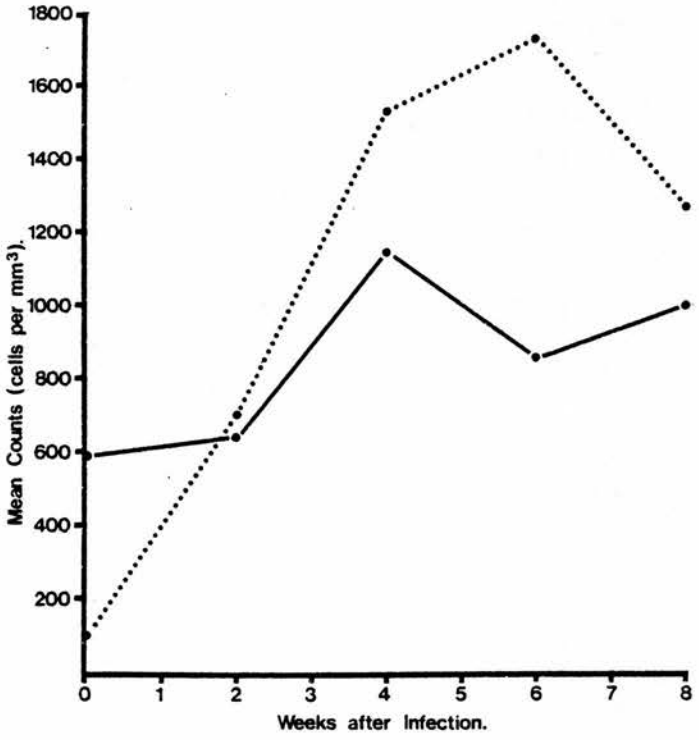
The immune response of rabbits to subcutaneous implantation with mature F. hepatica.

Rabbits group	challenge infection (metacercariae)	Nos. of flukes recovered at PM	
		Individual	mean ± s.d.
Implanted with 2, 3 or 5 mature flukes s/c (A)	100	11, 18, 27, 28, 35, 35, 44, 47	30.6 ± 12.3
Non-implanted (B)	100	12,30,36,41,41	32.0 ± 12.1

By Wilcoxon's two-sample test:

A v B, $P > 0.05$ n.s.

Fig.5·1. Peripheral Eosinophil Counts.



Group A —— Rats implanted S/C with living flukes before challenge.

Group B····· Infection control group.

Fig.5·2.

Implantation Experiment in Rats - Glutamic Dehydrogenase Levels.

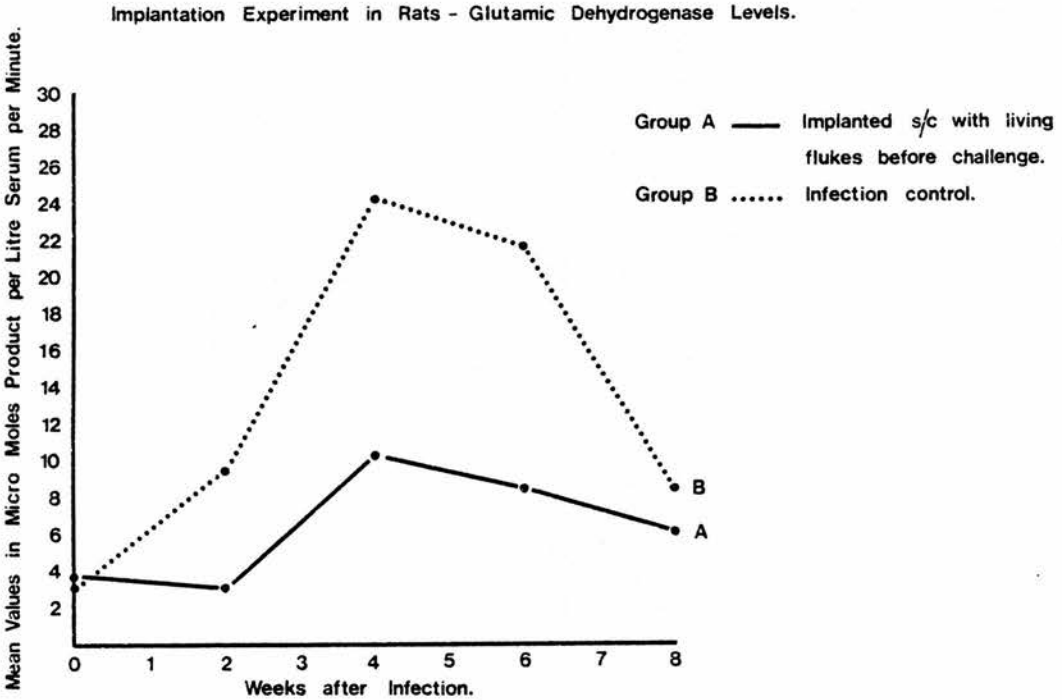


Fig. 5·3. Implantation Experiment in Rabbits-Peripheral Eosinophil Count.

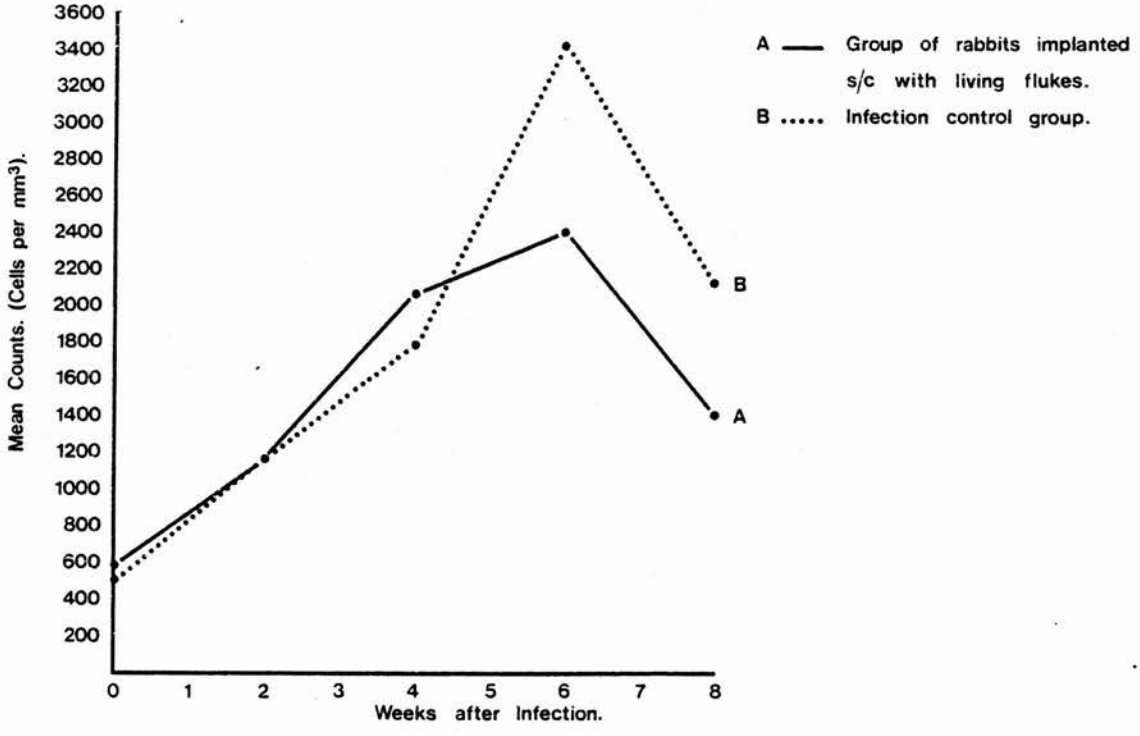
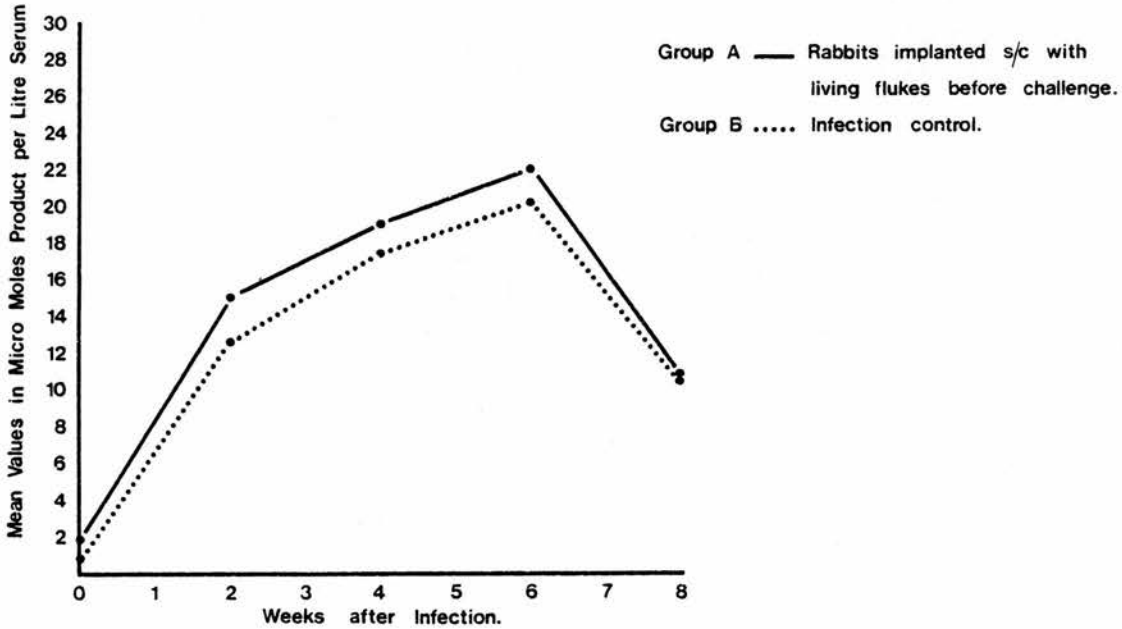


Fig. 5.4. Implantation Experiment in Rabbits - Glutamic Dehydrogenase Levels.



These findings confirm that resistance can be stimulated in rats in the absence of hepatic inflammation and fibrosis induced by primary infections. However, it is not possible at this stage to tell whether this resistance is stimulated by metabolic products, eggs from the mature flukes or by factors in the tegument of the fluke. The fact that subcutaneous implantation fails to stimulate resistance to challenge in rabbits supports the previous conclusion that the mechanical barrier which follows primary infection is probably responsible for any reduction in the number of flukes from challenge, where this occurs.

5.3. The effect of intraperitoneal implantation of mature flukes contained in diffusion chambers on the immune response of rats

Introduction

This experiment was carried out to see whether the metabolic products which are released from flukes will stimulate resistance to challenge in the absence of direct contact between the implanted flukes and the host tissues, thus excluding any effects due to the fluke tegument. At the same time it was designed to investigate the immune response due to direct intraperitoneal implantation of mature flukes. Since rabbits had shown no evidence of resistance due to subcutaneous implantation in the previous experiment, subsequent implantation experiments were undertaken only in rats.

A pilot experiment was first carried out to see whether the flukes inside diffusion chambers survive up to the time of

challenge i.e. 2 weeks after implantation. In this experiment diffusion chambers containing adult flukes were implanted intraperitoneally into 8 rats. Two of these were selected at random and killed at the 2nd, 5th, 7th and 14th day after implantation. The diffusion chambers were then taken out and examined. All the flukes were found to be alive. A diffusion chamber in the peritoneal cavity of a rat is shown in Plate 5.1.

Experimental design

Thirtynine rats were divided into 5 groups, A (12 rats), B (8 rats), C (7 rats), D (6 rats) and E (6 rats). Group A was implanted intraperitoneally with 1 or 2 living adult flukes in diffusion chambers and group B was similarly implanted with empty chambers. Group C was left as the unimplanted control group, while group D was implanted intraperitoneally with 1 or 2 dead flukes in chambers. Finally group E was implanted intraperitoneally with 1 or 2 un-encapsulated mature flukes. Serum was collected at days 2, 5, 7, 9, 11 and 14 after implantation to examine for precipitating antibodies. Rats in all groups were each challenged with 20 metacercariae of F. hepatica 2 weeks after implantation. Serum glutamic dehydrogenase activity was determined fortnightly and post-mortem examination was carried out 8 weeks after challenge.

Results

At post-mortem examination both the directly implanted flukes and the flukes in chambers were found to be dead and encysted.

Fluke recovery

This is shown in Table 5.3. A significant reduction in

Plate 5.1. A diffusion chamber in the peritoneal cavity of a rat.

TABLE 5.3.

The effect of intraperitoneal implantation of mature flukes in diffusion chambers on the immune response to *F. hepatica* by rats

Rats group	challenge infection (metacercariae)	Nos. of flukes recovered at PM	
		Individual	mean ± S.D.
Implanted I/P with 1 or 2 mature flukes in chambers (A)	20	1, 2, 2, 2, 3, 3, 4, 4, 4, 5, 5, 6	3.4 ± 1.5
Implanted I/P with 1 or 2 empty chambers (B)	20	3, 3, 5, 5, 6 7, 8, 10	5.9 ± 2.4
Non-implanted infection controls (C)	20	4, 7, 7, 8, 8 9, 12	7.9 ± 2.4
Implanted I/P with 1 or 2 dead flukes in chambers (D)	20	4, 5, 6, 6 6, 7	5.7 ± 1.0
Implanted I/P with 1 or 2 mature flukes (E)	20	1, 2, 3, 3, 4, 5	3.0 ± 1.4

By Wilcoxon's two-sample test:

A v B, $P<0.025$
A v C, $P<0.001$
A v D, $P<0.005$
E v C, $P<0.005$
A v E, $P>0.05$ ∴ n.s.
B v D, $P>0.05$ ∴ n.s.
B v C, $P>0.05$ ∴ n.s.

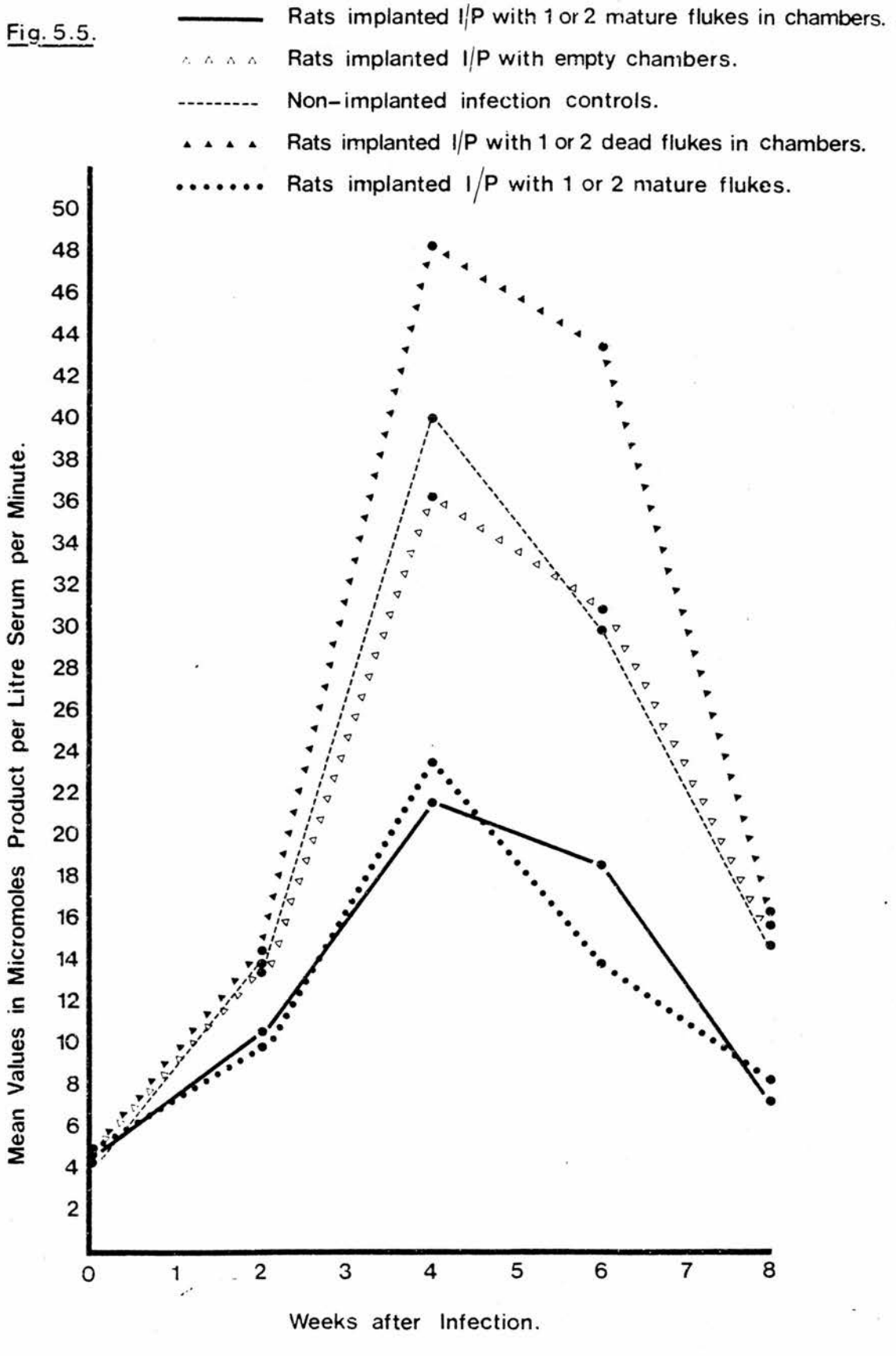
the numbers of flukes recovered from rats implanted with adult flukes in chambers was found compared to those from rats implanted with empty chambers, those from rats implanted with dead flukes in chambers, or those from non-implanted rats. Similarly, a significant reduction was found in the numbers of flukes recovered from rats directly implanted with adult flukes in the peritoneal cavity compared with those from non-implanted rats. However, no significant difference was found between the numbers of flukes recovered from rats implanted with flukes in chambers and from those implanted directly without encapsulation. Also no significant differences were found between the numbers of flukes recovered from rats implanted with dead flukes in chambers, from those implanted with empty chambers, or from non-implanted rats.

Serum glutamic dehydrogenase assays

These are recorded in Appendix Table 5.5. and Fig. 5.5. The enzyme levels in all groups progressively increased after infection reaching a peak at the 4th week. However, the peak levels were significantly lower in the groups implanted directly with living flukes or with living flukes in chambers than in the other three groups. (A v B at the 4th week, $t = 3.41$, $P<0.01$; at the 6th week, $t = 2.83$, $P<0.02$. A v C at the 4th week, $t = 3.38$, $P<0.01$; at the 6th week, $t = 2.67$, $P<0.02$. A v D at the 4th week, $t = 5.86$, $P<0.001$; at the 6th week, $t = 6.56$, $P<0.001$. E v C at the 4th week, $t = 2.74$, $P<0.02$; at the 6th week, $t = 3.13$, $P<0.01$. There were no significant differences in the mean enzyme levels of A v E, C v D, or B v C at any time).

The Effect of I/P Implantation of Mature Flukes in Diffusion Chambers on the Immunity to F.hepatica in Rats. Serum Glutamic Dehydrogenase Levels.

Fig. 5.5.



Serology

Precipitation lines in agar first appeared five days after implantation with living flukes whether or not the flukes were encapsulated in chambers. They did not appear after implantation with dead flukes in chambers.

Discussion

It is clear that mature F. hepatica implanted intraperitoneally or subcutaneously will stimulate an immune response which is protective to rats against challenge infections.

The fact that this protective immunological response was also found in rats implanted with living flukes in diffusion chambers (but not with dead flukes) indicates that this response can be stimulated by soluble metabolic products before the death of the flukes within the chambers. It also indicates that cellular contact between host and parasite is not necessary for the stimulation of resistance. Furthermore only a relatively small quantity of antigenic material is required, since resistance was stimulated by metabolic products from one or two flukes over a relatively short time.

5.4. The effect of subcutaneous implantation and removal of mature flukes in diffusion chambers on the immune response to F. hepatica by rats

Introduction

This experiment was carried out to confirm that the metabolic products from living flukes released into the subcutaneous tissues over as little as two weeks are capable of stimulating resistance to challenge.

Experimental design

Fortytwo rats were divided into 6 groups, A (8 rats), B (9 rats), C (5 rats), D (5 rats), E (8 rats) and F (7 rats). Rats in group A were implanted subcutaneously with 2 mature flukes in chambers and group B was similarly implanted with empty chambers. Group C was implanted subcutaneously with mature flukes in chambers and group D was similarly implanted with empty chambers. Similarly group E was implanted intraperitoneally with mature flukes in chambers and group F was implanted with empty chambers. Two weeks later the chambers were removed from the rats in groups A and B. The rats in the six groups were then each challenged with 20 metacercariae of F. hepatica. Serum glutamic dehydrogenase activity was determined fortnightly and post-mortem examination of all six groups was carried out 8 weeks after challenge.

Results

At post-mortem examination the chambers in the subcutaneous tissues and intraperitoneal cavity were encysted and all flukes inside them were dead. In contrast the flukes present in the chambers removed from the rats in group A, 2 weeks after implantation, were all still alive.

Fluke recovery

The numbers of flukes recovered from the rats are shown in Table 5.4. A significant reduction was found in the numbers recovered from the rats in group A compared with those from group B. Significant reductions were also found in the fluke burdens in group C and E when compared with those recovered in the control groups D and F respectively. In

TABLE 5.4.

The effect of subcutaneous implantation and removal of mature flukes in diffusion chambers on the immune response to F. hepatica by rats.

Rats group	challenge infection (metacercariae)	Nos. of flukes recovered at p.m.	
		Individual	mean ± S.D.
Implanted S/C with 2 mature flukes in chambers for 2 weeks and removed before challenge (A)	20	0, 0, 0, 0, 1, 1, 3, 3	1.0 ± 1.3
Implanted S/C with 2 empty chambers for 2 weeks and removed before challenge (B)	20	1, 2, 2, 3, 3, 3, 4, 4, 5	3.0 ± 1.2
Implanted S/C with 2 mature flukes in chambers for 2 weeks before challenge (C)	20	0, 1, 1, 2, 3	1.4 ± 1.1
Implanted with 2 empty chambers S/C for 2 weeks before challenge (D)	20	2, 2, 3, 4, 5	3.2 ± 1.3
Implanted I/P with 2 mature flukes in chambers for 2 weeks before challenge (E)	20	0, 0, 0, 1 1, 1, 2, 3	1.0 ± 1.1
Implanted I/P with 2 empty chambers for 2 weeks before challenge (F)	20	1, 2, 3, 3, 4, 4, 4	3.0 ± 1.2

By Wilcoxon's two-sample test:

A v B, $P < 0.01$ E v F, $P < 0.005$ A v E, $P > 0.05$, n.s. B v F $P > 0.05$, n.s.
C v D, $P < 0.05$ A v C, $P > 0.05$, n.s. B v D $P > 0.05$ n.s.

contrast there were no significant differences between groups A, C and E and between groups B, D and F respectively.

Serum glutamic dehydrogenase assays

These are recorded in Appendix Table 5.6. and Fig. 5.6. The enzyme levels in all groups progressively increased after infection to a peak at the 4th or 6th weeks. However the peaks were significantly lower in the groups of rats implanted with living flukes compared with the controls (A v B at the 4th week after infection, $t = 7.47$, $P < 0.001$; at the 6th week, $t = 5.56$, $P < 0.001$. C v D at the 4th week, $t = 2.41$, $P < 0.05$; at the 6th week, n.s. E v F at the 4th week, $t = 5.93$, $P < 0.001$; at the 6th week, $t = 5.39$, $P < 0.001$. There were no significant differences between the enzyme levels of A v C, A v E, B v D, B v F or D v F at any time.

Discussion

The results of this experiment will be discussed with those of the next experiment.

5.5. The effect of repeated intraperitoneal implantations of mature flukes encapsulated in diffusion chambers on the immune response to *F. hepatica* by rats

Introduction

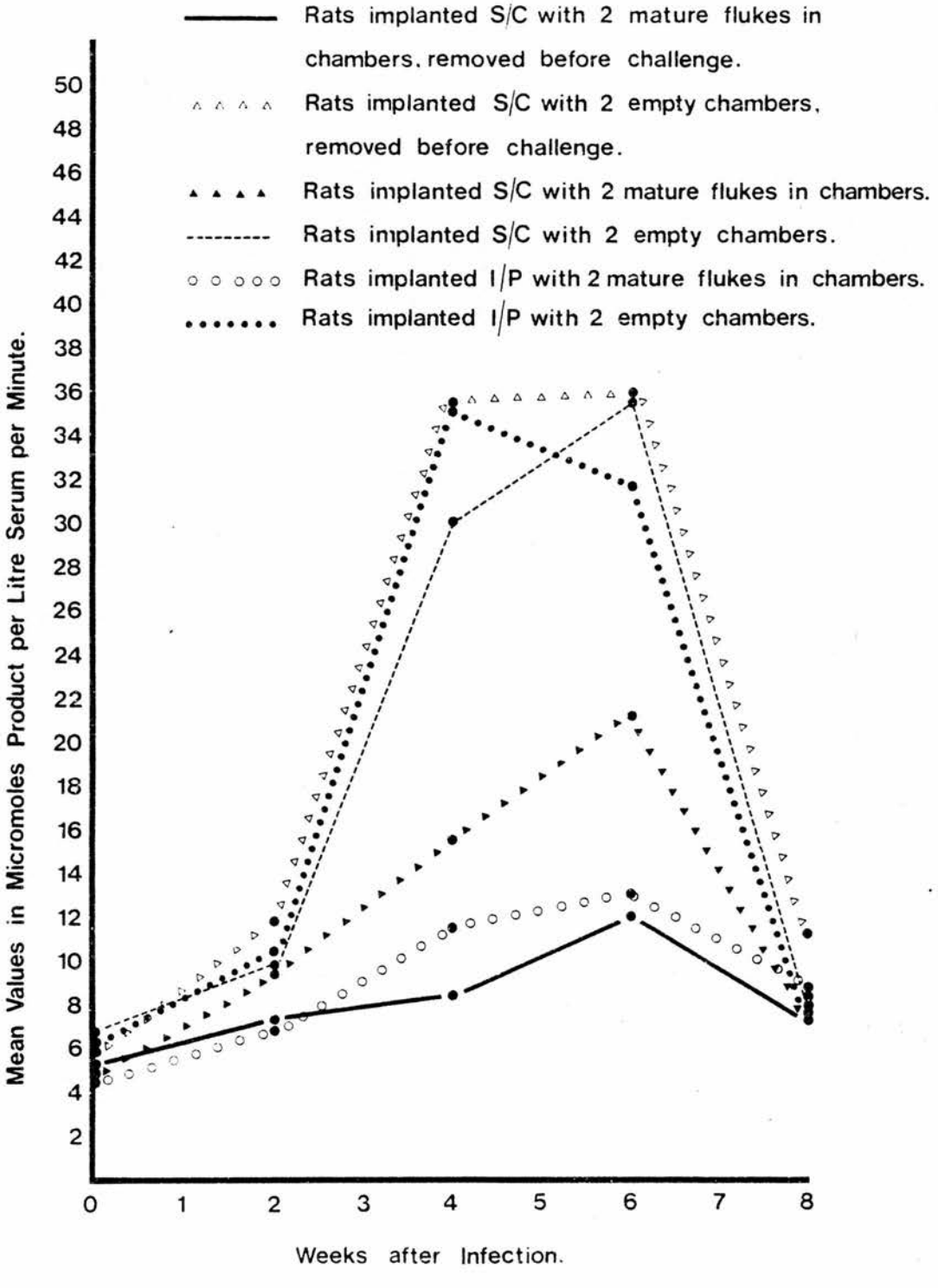
This experiment was carried out to see whether the level of resistance can be increased by repeating the immunogenic stimulus.

Experimental design

Thirteen rats were divided into two groups A (7 rats) and B (6 rats). Group A was implanted intraperitoneally with single mature flukes in chambers at the same time as group E of the

Fig. 5.6.

The Effect of S/C Implantation and Removal of Mature Flukes in Chambers on the Immunity to F.hepatica in Rats. Serum Glutamic Dehydrogenase Levels.



previous experiment (5.4.). Group B was similarly implanted with empty chambers. Similar chambers with or without living flukes were implanted again in the respective group 12 weeks later. Two weeks after these second implantations each rat in both groups was infected with 20 metacercariae of F. hepatica. Serum was examined weekly after the first and second implantations for precipitating antibodies. Serum glutamic dehydrogenase activity was determined fortnightly after challenge and post-mortem examination was carried out after 8 weeks.

Results

At post-mortem examination the chambers were found within cysts and the flukes were dead.

Fluke recovery

This is shown in Table 5.5. A very highly significant reduction was found in the numbers of flukes recovered from the rats in group A compared with those in group B. The level of resistance (91%) in group A was higher than that in group E (67%) of the previous experiment (5.4) in which rats were implanted on one occasion and challenged after 2 weeks.

Serum glutamic dehydrogenase assays

These are recorded in Appendix Table 5.7. and Fig. 5.7. The level of the enzyme in the control group showed a progressive elevation following infection reaching a peak at the 6th week. However, in the implanted group there was only a slight increase at the 6th week in one rat, the enzyme levels in the others being within the normal range. There

TABLE 5.5.

The effect of repeated intraperitoneal implantations of mature flukes encapsulated in diffusion chambers on the immune response to *F. hepatica* by rats.

Rats group	challenge infection (metacercariae)	Nos. of flukes recovered at p.m.	
		Individual	mean ± S.D.
Implanted I/P with 2 mature flukes in chambers twice at an interval of 12 weeks before challenge (A)	20	0, 0, 0, 0 0, 0, 1	0.14 ± 0.37
Implanted I/P with 2 empty chambers twice at an interval of 12 weeks before challenge (B)	20	1, 2, 2, 3 3, 6	2.8 ± 1.72

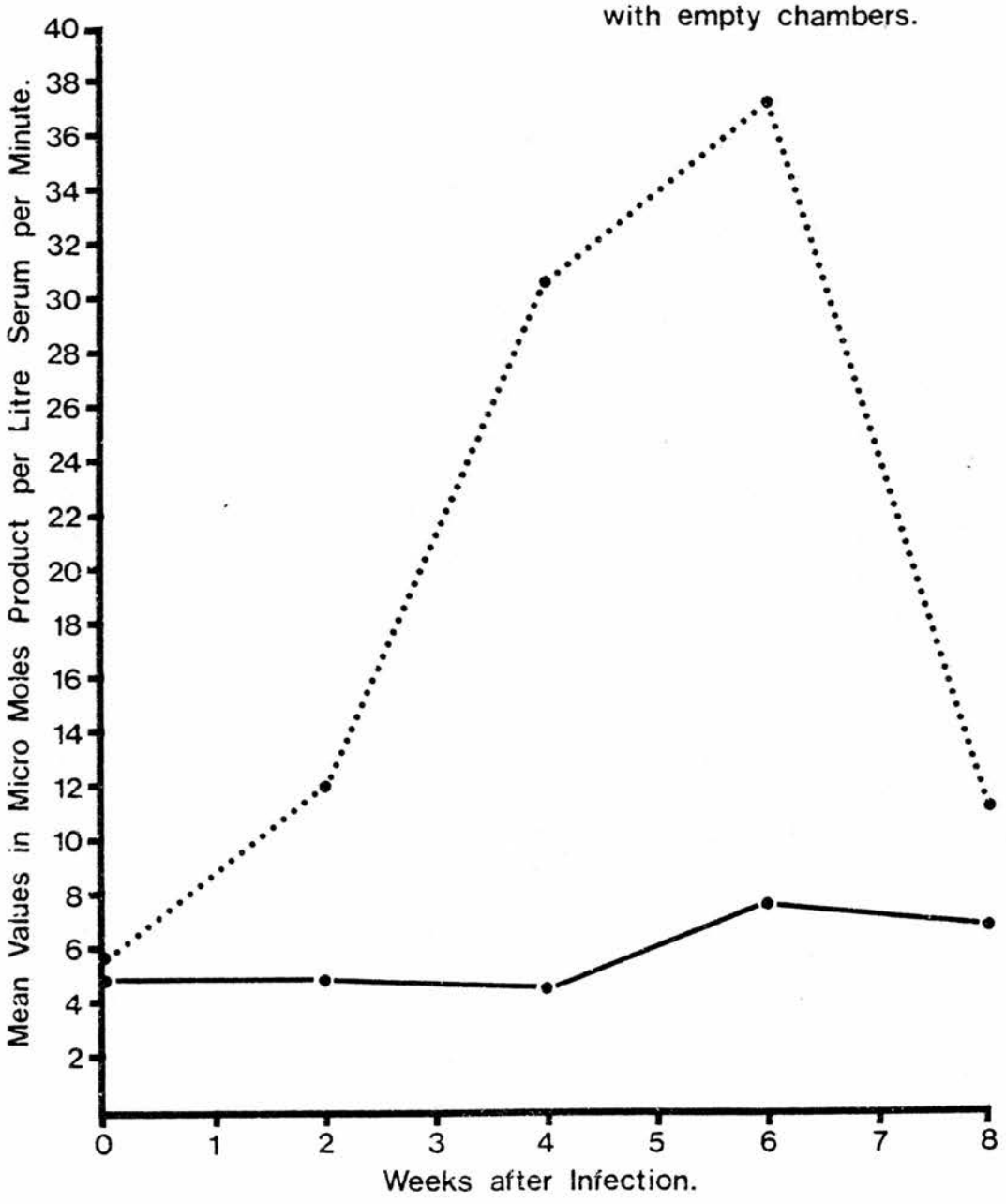
By Wilcoxon's two sample test:

A v B, $P < 0.001$

Fig. 5·7. Serum Glutamic Dehydrogenase Assays.

Group A ——— Rats implanted I/P with 2 mature flukes in chambers twice at an interval of 12 weeks before challenge

Group B..... Controls similarly implanted with empty chambers.



was a highly significant difference between the mean enzyme levels of the implanted and control groups at the 4th and 6th weeks after infection (A v B at the 4th week after infection, $t = 8.75$, $P < 0.001$; at the 6th week, $t = 8.68$, $P < 0.001$).

Serology

Precipitating antibodies were detectable in all rats in Group A by the week after the first implantation and continued to be detected up to the 11th week. In the 12th week they could be detected only in the serum of one rat. However, a week after the second implantation the sera of all implanted rats again contained precipitating antibodies.

Discussion

The fact that resistance could be induced by implanting single flukes in diffusion chambers and removing them within 2 weeks confirms that only small amounts of metabolic products are necessary to stimulate a significant resistance to challenge. This resistance develops whether the antigens are released into the subcutaneous tissues or into the peritoneal cavity. It has also been shown that the serological response diminishes with time and may no longer be detectable after 12 weeks. However, it can be restored by restimulation with the antigen and this also appears to result in a higher level of resistance than that initiated by the initial stimulus. In this respect, the experiment was not strictly controlled (as the challenge infections were not given at the same time or from the same pool of metacercariae) but the level of resistance (91%) obtained following the double implantation procedure was greater than that recorded in any of the previous implantation experiments.

CHAPTER 6

STUDIES ON THE PASSIVE TRANSFER OF RESISTANCE TO FASCIOLA HEPATICA IN RATS AND RABBITS

Introduction

It was concluded from the work described in the previous chapters that soluble immunologically active factors play a role in the resistance of rats to F. hepatica although no evidence for this was found in rabbits. However, these factors could be stimulating either a humoral or a cellular response, or perhaps, both are involved. Experiments were therefore designed to study the protective effect of circulating antibodies passively transferred into rats and rabbits.

6.1. Passive transfer of resistance to F. hepatica in rats by immune homologous serum

Experimental design

Twentysix rats were divided into 3 groups, A (12 rats), B, (8 rats) and C (6 rats). Each rat was infected with 20 metacercariae of F. hepatica. Immediately after infection the rats in group A were each injected intraperitoneally with 10 ml of immune serum from rats which had been infected with 20 metacercariae 8 weeks previously. This was repeated 2 days later. Group B was similarly twice injected with normal homologous serum, while group C was left as the challenge control. Peripheral eosinophil counts and serum glutamic dehydrogenase activities were determined fortnightly and necropsy was carried out 8 weeks after infection.

Results

Fluke recovery

This is recorded in Table 6.1. A highly significant difference was found between the numbers of flukes recovered from rats injected with immune serum compared with those from rats injected with normal serum ($P < 0.01$). Furthermore, the difference between the numbers of flukes recovered from the rats in the former group and those from the challenge controls was even greater ($P < 0.001$). However, the numbers of flukes recovered from rats injected with normal serum and those from challenge controls were also significantly different at $P < 0.05$.

Peripheral eosinophil counts

These are shown in Appendix Table 6.1. and Fig. 6.1. The eosinophil counts in rats injected with normal serum and those in challenge controls showed similar progressive increases peaking at the 6th week. However, the counts in rats injected with immune serum showed little further increase after the initial rise 2 weeks after infection.

Serum glutamic dehydrogenase assays

The results are shown in Appendix Table 6.2. and Fig. 6.2. Infection was followed by similar elevations of the enzyme levels in the group injected with normal serum and in the challenge controls, with slightly higher levels in the former. In contrast there was very little increase after infection in the group injected with immune serum.

TABLE 6.1.

Passive transfer of resistance to *F. hepatica* in rats by immune homologous serum.

Rats group	challenge infection (metacercariae)	Nos. of flukes recovered at p.m.	
		Individual	mean ± S.D.
2 x 10 ml immune rat serum I/P at days 0 and 2(A)	20	0, 0, 0, 0, 0 0, 0, 0, 0, 1 1, 3	0.4 ± 0.9
Challenge controls (B)	20	1, 1, 2, 2, 3, 4, 4, 5	2.8 ± 1.5
2 x 10 ml normal rat serum I/P at days 0 and 2(C)	20	1, 1, 1, 1, 2, 2	1.3 ± 0.5

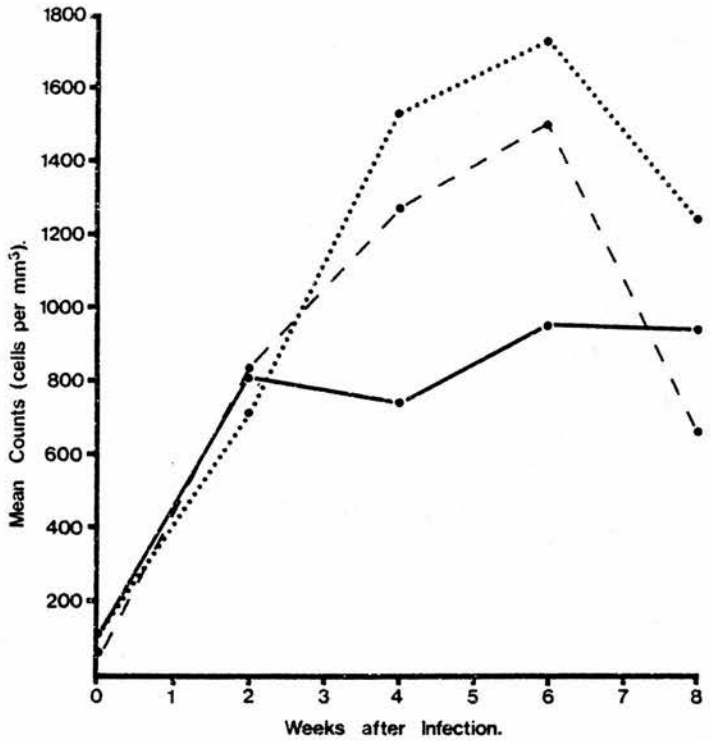
By Wilcoxon's two-sample test:

A v B, $P < 0.001$

A v C, $P < 0.01$

B v C, $P < 0.05$

Fig. 6·1. Peripheral Eosinophil Counts.

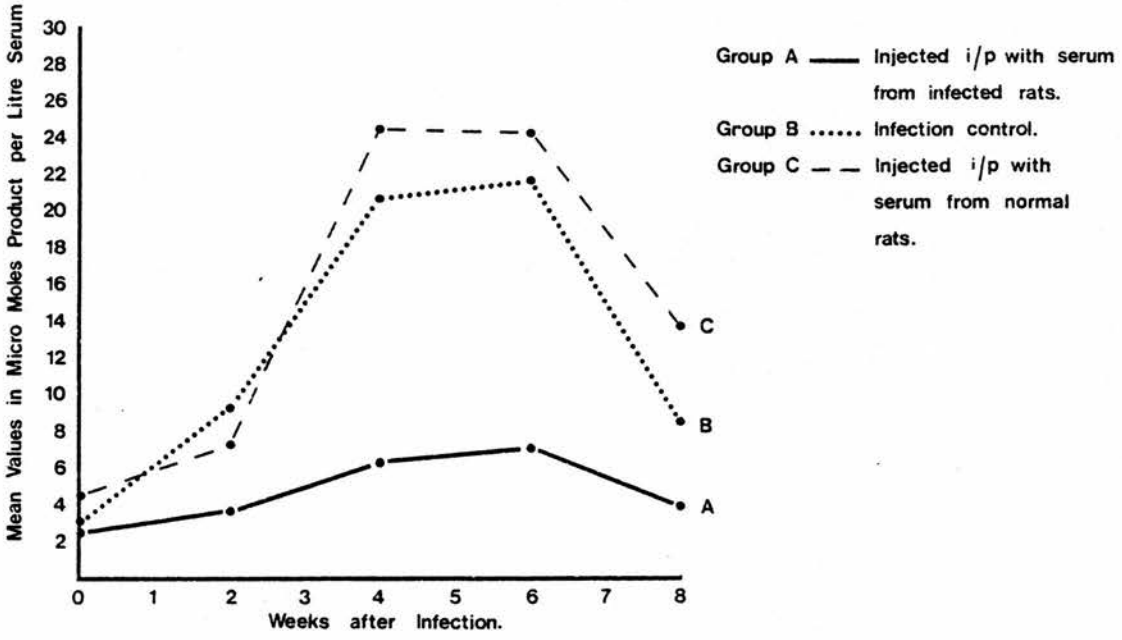


Group A ——— Rats injected I/P with serum from infected rats.

Group B ..... Infection control.

Group C — — Rats injected I/P with serum from normal rats.

Fig. 6.2. Serum Transfer Experiment in Rats - Glutamic Dehydrogenase Levels.



The mean enzyme levels of the rats in the group given the immune serum were significantly lower than those of the rats in either of the other two groups at 4 and 6 weeks after infection (At 4 weeks, A v B, $t = 4.60$, $P < 0.001$; A v C, $t = 4.27$, $P < 0.001$; at 6 weeks, A v B, $t = 3.67$, $P < 0.01$; A v C, $t = 3.56$, $P < 0.01$). On the other hand, no significant difference was found between the enzyme levels of the two control groups B and C at any time.

Discussion

Although it is clear that homologous immune serum can confer a significant resistance against F. hepatica in rats, it appears from the apparent resistance conferred even by normal serum that a 'flushing effect' of serum on the young flukes in the peritoneum contributed to this resistance. However, as the normal serum did not confer a similar level of resistance, this suggests that the reduction in the flukes recovery was not simply due to this 'flushing effect' and tends to confirm that circulating antibodies have a protective effect.

6.2. Passive transfer of resistance to F. hepatica in rabbits by immune homologous serum

Experimental design

Fourteen rabbits were divided into 3 groups, A (5 rabbits), B (5 rabbits) and C (4 rabbits). The rabbits were each infected with 100 metacercariae of F. hepatica. This was immediately followed by intraperitoneal injection of each rabbit in group A with 24 ml of immune serum obtained from rabbits which had been infected with 100 metacercariae 8

weeks previously. These injections were repeated 2 days later. Group B was similarly injected with normal homologous serum and group C was left as the challenge control group. Peripheral eosinophil counts and glutamic dehydrogenase assays were determined fortnightly and post-mortem examination was carried out after 10 weeks.

Results

Fluke recovery

This is recorded in Table 6.2. No significant differences were found between the numbers of flukes recovered from rabbits injected with immune serum compared to those from rabbits injected with normal serum or from the challenge controls.

Peripheral eosinophil counts

These are shown in Appendix Table 6.3. and Fig. 6.3. The counts peaked at the 6th week after infection in the control groups and at the 8th week in the group injected with immune serum. Higher counts were observed in rabbits injected with normal or immune serum than in challenge controls.

Serum glutamic dehydrogenase assays

The results are recorded in Appendix Table 6.4. and Fig. 6.4. The enzyme levels were similar in all three groups with no significant differences between the mean levels at any time.

Discussion

In contrast to the findings in rats, immune homologous serum failed to confer resistance in rabbits. This may have been either because immune rabbit serum is not protective or

TABLE 6.2.

Passive transfer of resistance to F. hepatica in rabbits by immune homologous serum.

Rabbits group	challenge infection (metacercariae)	Nos. of flukes recovered at p.m.	
		Individual	mean ± S.D.
2 x 24 ml immune rabbit serum I/P at days 0 and 2 (A)	100	19, 21, 27, 54, 58	35.8 ± 18.7
2 x 24 ml normal rabbit serum I/P at days 0 and 2 (B)	100	19, 34, 37 40, 51	36.2 ± 11.6
Challenge controls (C)	100	32, 35, 40 44	37.8 ± 5.3

By Wilcoxon's two-sample test:

A v B, $P > 0.05$, n.s.

A v C, $P > 0.05$, n.s.

Fig. 6.3. Serum Transfer Experiment in Rabbits - Peripheral Eosinophil Count.

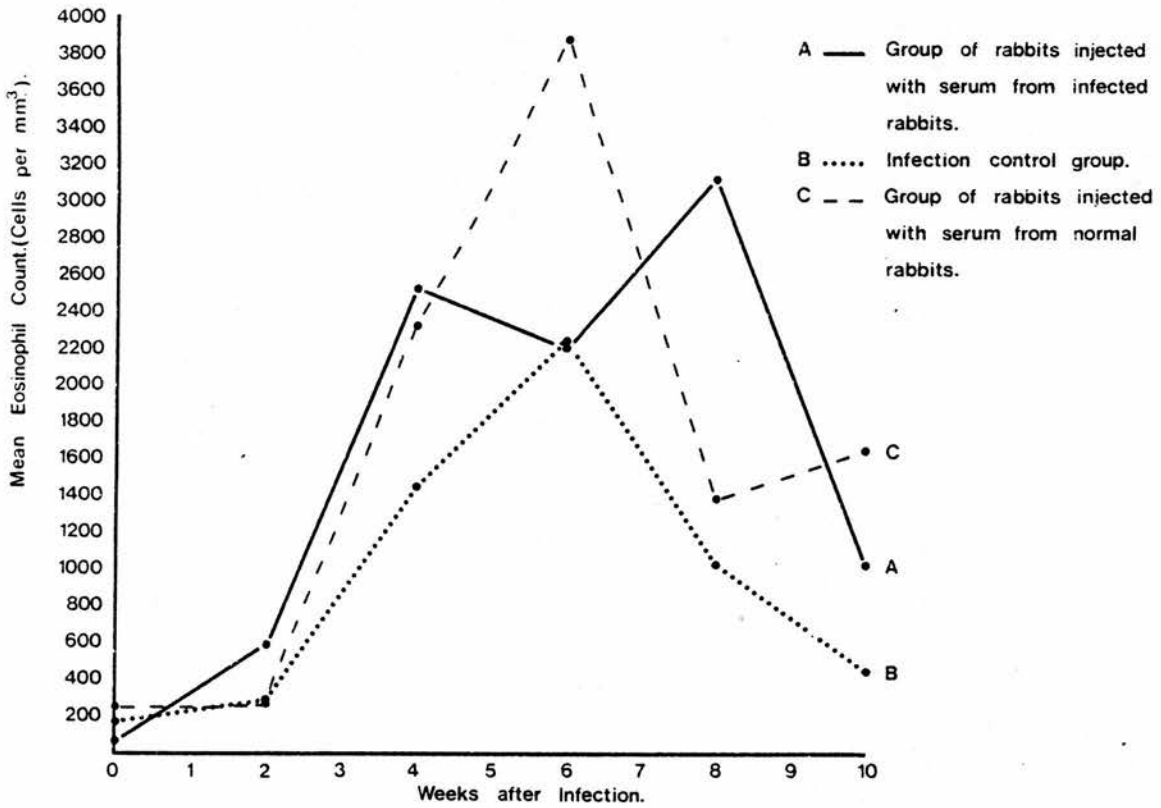
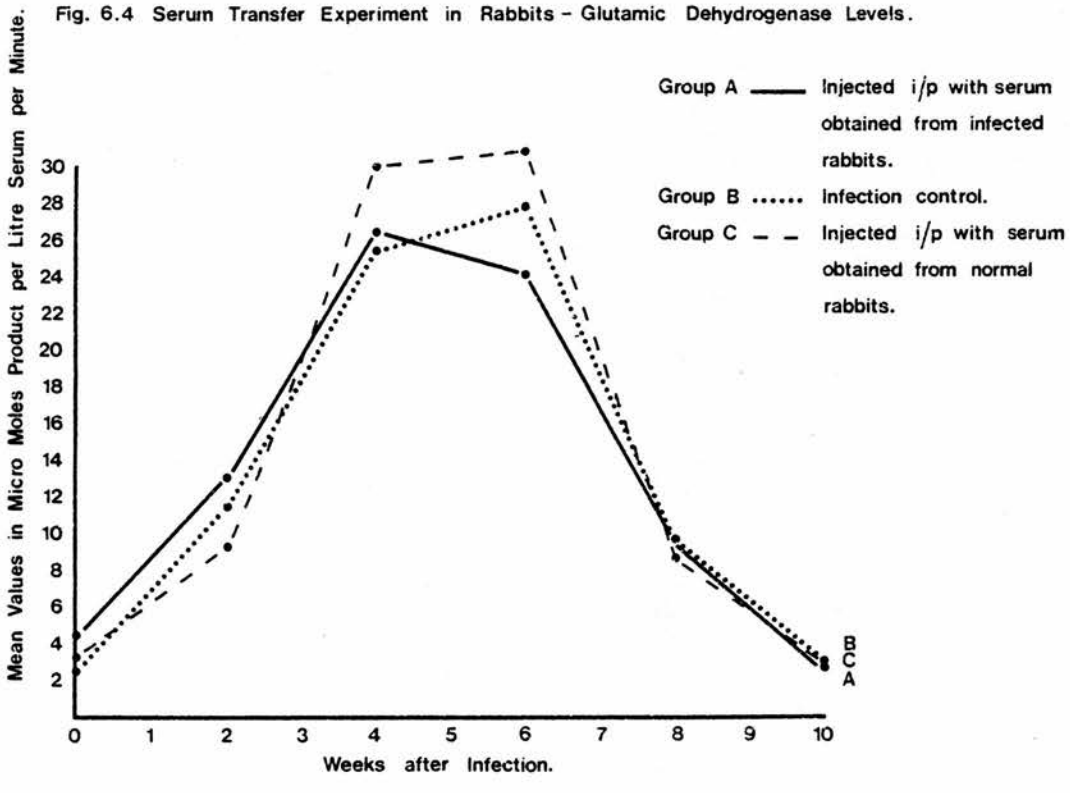


Fig. 6.4 Serum Transfer Experiment in Rabbits - Glutamic Dehydrogenase Levels.



because the volume of serum was not sufficiently large to confer resistance. The ratio of the transferred serum to recepient weight was about 1:50 in rabbits compared to about 1:15 in rats, which may also account for the fact that the administration of normal rabbit serum had no effect on the fluke burdens in the rabbits similar to that seen in the rats.

6.3. The effect of intraperitoneal transfer of immune rat serum on resistance to F. hepatica by rabbits

Introduction

This experiment was carried out to see whether immune rat serum could protect rabbits against F. hepatica.

Experimental design

Immune serum was collected from 60 rats which had been infected 8-10 weeks earlier with 20 metacercariae of F. hepatica.

Three groups of rabbits (A, B and C) and 2 groups of rats (D and E) were used. There were 7 rabbits in group A, 4 rabbits in group B, 8 rabbits in group C, 6 rats in group D and 5 rats in group E. The rabbits were each infected with 100 metacercariae while the rats were each infected with 20 metacercariae. Each rabbit in group A was injected intraperitoneally with 25 ml of immune rat serum immediately after infection and this was repeated 2 days later. The rabbits in group B were similarly injected with normal rat serum, while those in group C were left as the challenge controls. The rats in group D were each injected intraperitoneally immediately after infection with 10 ml from the same pool of immune serum as was used for the rabbits in group A and this was also repeated after 2 days. This group was used as a control for the

protective value of the immune rat serum. Group E was similarly injected but with normal rat serum from the same pool as was used in the rabbits in group B. Peripheral eosinophil counts and serum glutamic dehydrogenase activities were determined fortnightly and post-mortem examinations were carried out 8 weeks after challenge infection.

Results

Fluke recovery

This is recorded in Table 6.3. A significant difference was found between the numbers of flukes recovered from the rabbits injected with immune rat serum compared with those from the rabbits injected with normal rat serum or from the challenge controls. A significant difference was also found between the numbers of flukes recovered from the rats injected with immune rat serum and those from the rats injected with normal rat serum. On this occasion the administration of normal rat serum appeared to reduce the number of flukes which were recovered from the recepient rabbits compared with challenge controls but this difference was not significant.

Peripheral eosinophil counts

These are recorded in Appendix Table 6.5., Fig. 6.5.1. and Fig. 6.5.2. The counts showed similar trends in all three groups of rabbits, peaking at the 6th week. However, counts from rabbits injected with normal or immune serum were higher than those from challenge controls, the highest peak being shown by the rabbits injected with normal rat serum.

TABLE 6.3.

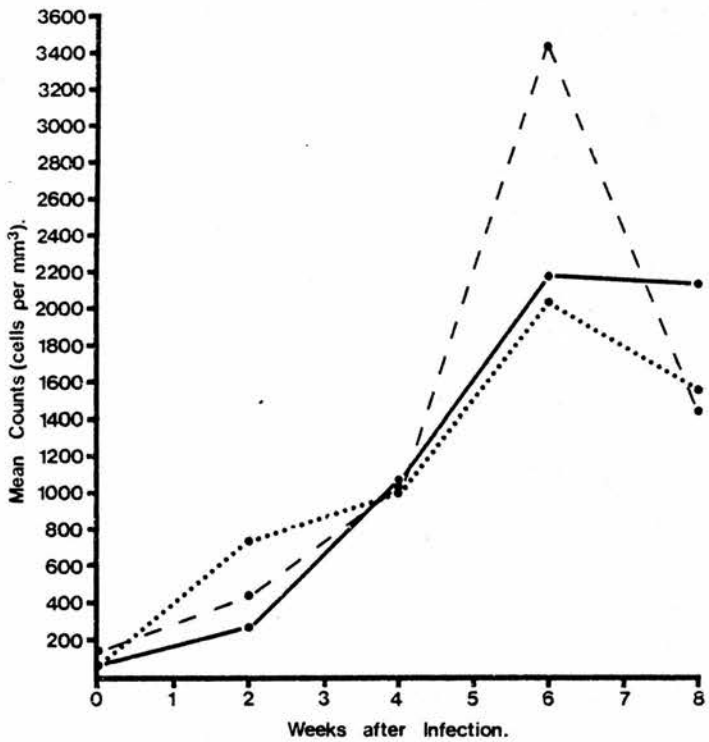
The effect of intraperitoneal transfer of immune rat serum on resistance to F. hepatica by rabbits.

Group	challenge infection (metacercariae)	Nos. of flukes recovered at p.m.	
		Individual	mean ± S.D.
Rabbits injected I/P with 2 x 25 ml immune rat serum at days 0 and 2 (A)	100	3, 7, 9, 20, 25, 33, 35	16.5 ± 13.6
Rabbits injected I/P with 2 x 25 ml normal rat serum at days 0 and 2 (B)	100	29, 29, 37, 38	33.3 ± 4.9
Challenge controls (C)	100	32, 32, 36, 41 44, 49, 53, 58	43.1 ± 9.7
Rats injected I/P with 2 x 10 ml immune rat serum at days 0 and 2 (D)	20	0, 1, 1, 1, 2, 2	1.2 ± 0.8
Rats injected I/P with 2 x 10 ml of normal rat serum at days 0 and 2 (E)	20	2, 3, 5, 5, 7	4.4 ± 2.0

By Wilcoxon's two-sample test:

A v B, $P < 0.05$ D v E, $P < 0.005$
A v C, $P < 0.005$ B v C, $P > 0.05$, n.s.

Fig. 6·51. Peripheral Eosinophil Counts.

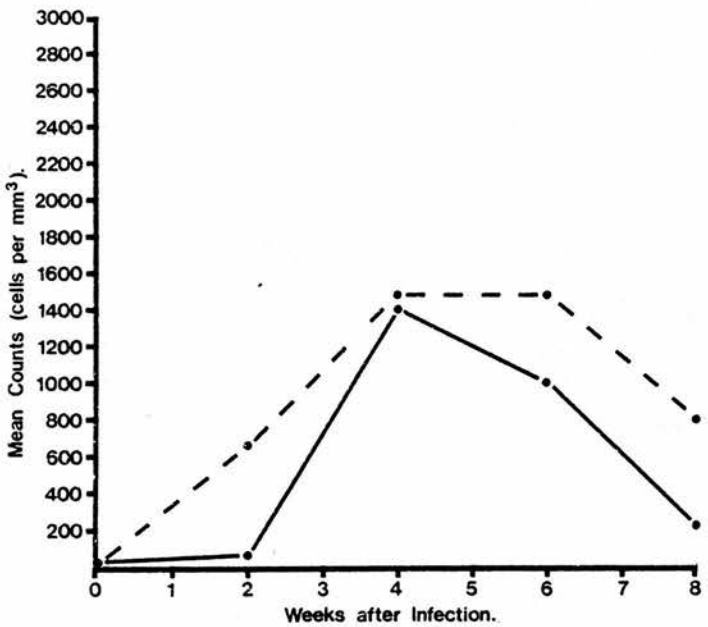


Group A ——— Rabbits injected with immun rat serum.

Group B — — Rabbits injected with normal rat serum.

Group C ..... Infection controls.

Fig.6·5·2. Peripheral Eosinophil Counts.



Group D—— Rats injected with immune rat serum.

Group E— — Rats injected with normal rat serum.

Rats in the group injected with immune serum and those in the group injected with normal rat serum showed increased counts which peaked at the 4th week in the former and at the 6th week in the latter.

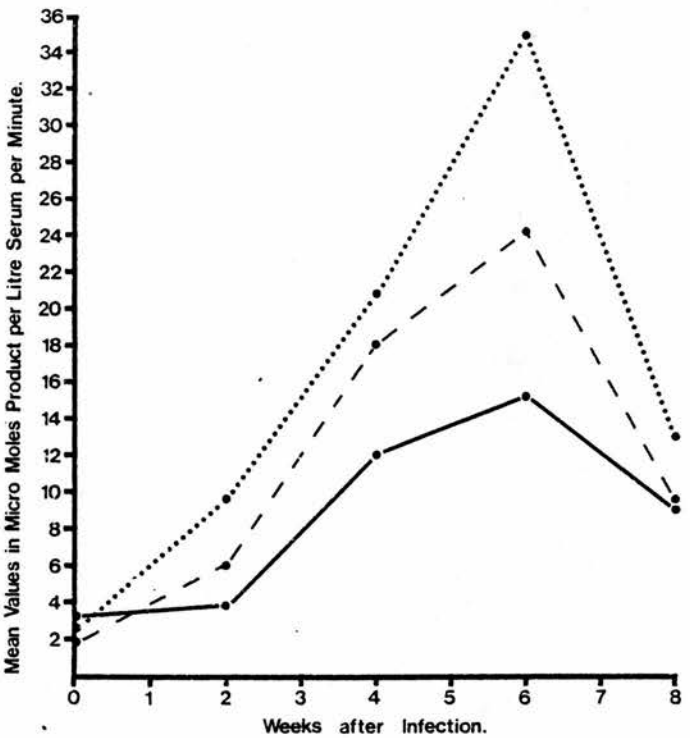
Serum glutamic dehydrogenase assays

The results are shown in Appendix Table 6.6., Fig. 6.6.1. and Fig. 6.6.2. The enzyme levels increased after infection in all 3 groups of rabbits reaching a peak at the 6th week. However, the challenge controls showed the highest peak while the rabbits injected with immune rat serum showed the lowest peak. The difference between the mean enzyme activity in the rabbits injected with immune rat serum and that in the rabbits injected with normal rat serum was significant at the 4th week ($t = 2.61$, $P < 0.05$) but not at the 6th week. Furthermore, the differences between the mean enzyme activity of rabbits injected with immune rat serum and the challenge controls were even greater at the 4th week ($t = 3.19$, $P < 0.01$) and the 6th week ($t = 3.44$, $P < 0.01$). There was no significant difference in the mean enzyme activity of the rabbits injected with normal rabbit serum and that of the challenge controls. On the other hand, a highly significant difference was found between the mean enzyme activity of rats injected with immune rat serum compared to that of rats injected with normal rat serum at the 4th week ($t = 9.48$, $P < 0.001$) but not at the 6th week after infection.

Discussion

Immune rat serum which conferred resistance on recepient rats was found to be protective to rabbits as well. A similar

Fig.6·6·1. Serum Glutamic Dehydrogenase Levels.

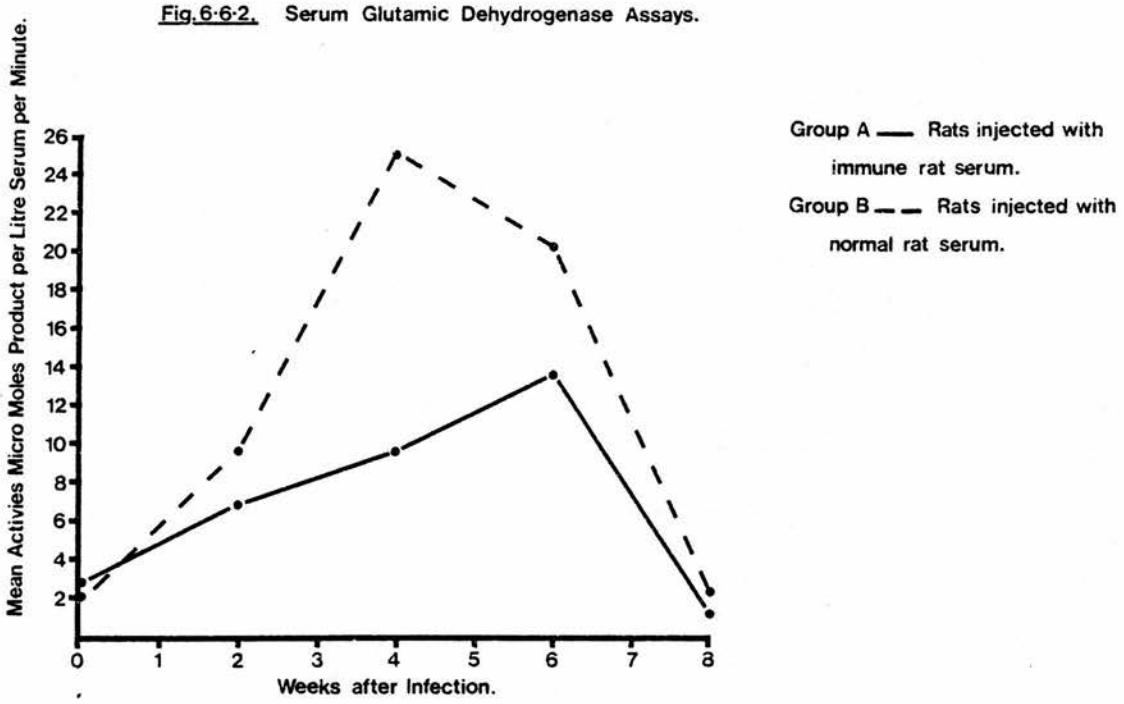


Group A ___ Rabbits injected with immune rat serum.

Group B _ _ Rabbits injected with normal rat serum.

Group C..... Infection controls.

Fig.6·6·2. Serum Glutamic Dehydrogenase Assays.



Group A —— Rats injected with immune rat serum.

Group B — — Rats injected with normal rat serum.

volume of immune rabbit serum had previously failed to protect rabbits against F. hepatica. This is probably due to a greater protective capacity of immune rat serum. However, the great individual variation which resulted in some of the rabbits which had received the immune rat serum being very resistant while others showed little or no resistance probably indicates that this is rather a disparate ability in rabbits.

6.4. The effect of intraperitoneal transfer of immune rabbit serum on resistance to F. hepatica by rats

Introduction

This experiment was carried out to see whether immune rabbit serum can confer resistance to recepient rats although it fails to do so in recepient rabbits.

Experimental design

Donor rabbits had 8-week-old infections with 100 metacercariae of F. hepatica.

Two groups (A and B) each of 5 rats were used. Each rat was infected with 20 metacercariae. Immediately after infection the rats in group A were each injected intraperitoneally with 10 ml of immune rabbit serum and this was repeated after 2 days. Group B was similarly injected with normal rabbit serum. Peripheral eosinophil counts and serum glutamic dehydrogenase activities were determined fortnightly and necropsy was carried out 8 weeks after infection.

Results

Fluke recovery

This is recorded in Table 6.4. A significant difference was found between the numbers of flukes recovered from the

TABLE 6.4.

The effect of intraperitoneal transfer of immune rabbit serum on resistance to *F. hepatica* by rats.

Rats group	challenge infection (metacercariae)	Nos. of flukes recovered at p.m.	
		Individual	mean ± S.D.
2 x 10 ml immune rabbit serum I/P on days 0 and 2 (A)	20	0, 0, 1, 1, 2	0.8 ± 0.8
2 x 10 ml normal rabbit serum I/P on days 0 and 2 (B)	20	1, 2, 3, 4, 4	2.8 ± 1.3

By Wilcoxon's two sample test:

A v B, $P < 0.05$

two groups.

Peripheral eosinophil counts

These are shown in Appendix Table 6.7. and Fig. 6.7. Infection was followed by similar increased eosinophil counts which reached a peak at the 6th week in both groups.

Serum glutamic dehydrogenase assays

The results are recorded in Appendix Table 6.8. and Fig. 6.8. The enzyme levels in the rats injected with normal rabbit serum rose to a peak at the 6th week, but the elevation in the rats injected with immune rabbit serum was less. There was a highly significant difference between the enzyme levels of the two groups at the 4th week ($t = 5.66$, $P < 0.001$) and the 6th week ($t = 5.20$, $P < 0.001$) after infection.

Discussion

Although immune rabbit serum failed to confer protection on rabbits (Experiment 6.2.), it did appear to protect recepient rats. This may have been because the ratio of the transferred serum to the body weight of the recepient was about 1:15 in rats and 1:50 in rabbits. It is therefore possible that a larger volume of immune rabbit serum is required to confer protection in rabbits. If this is so, the fact that in the earlier experiments immune rat serum was able to confer resistance on rabbits while a similar volume of immune rabbit serum had failed to do so, supports the previous suggestion that the protective capacity of the former is greater than that of the latter.

Fig.6·7. The Effect of I/P Transfer of Immune Rabbit Serum on the Immunity to F.hepatica in Rats. Peripheral Eosinophil Counts.

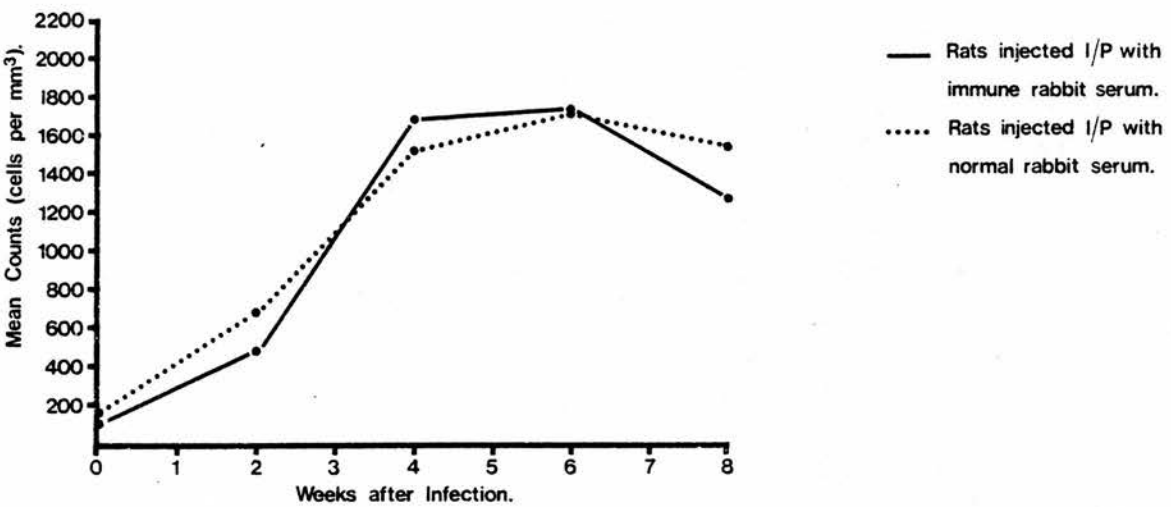
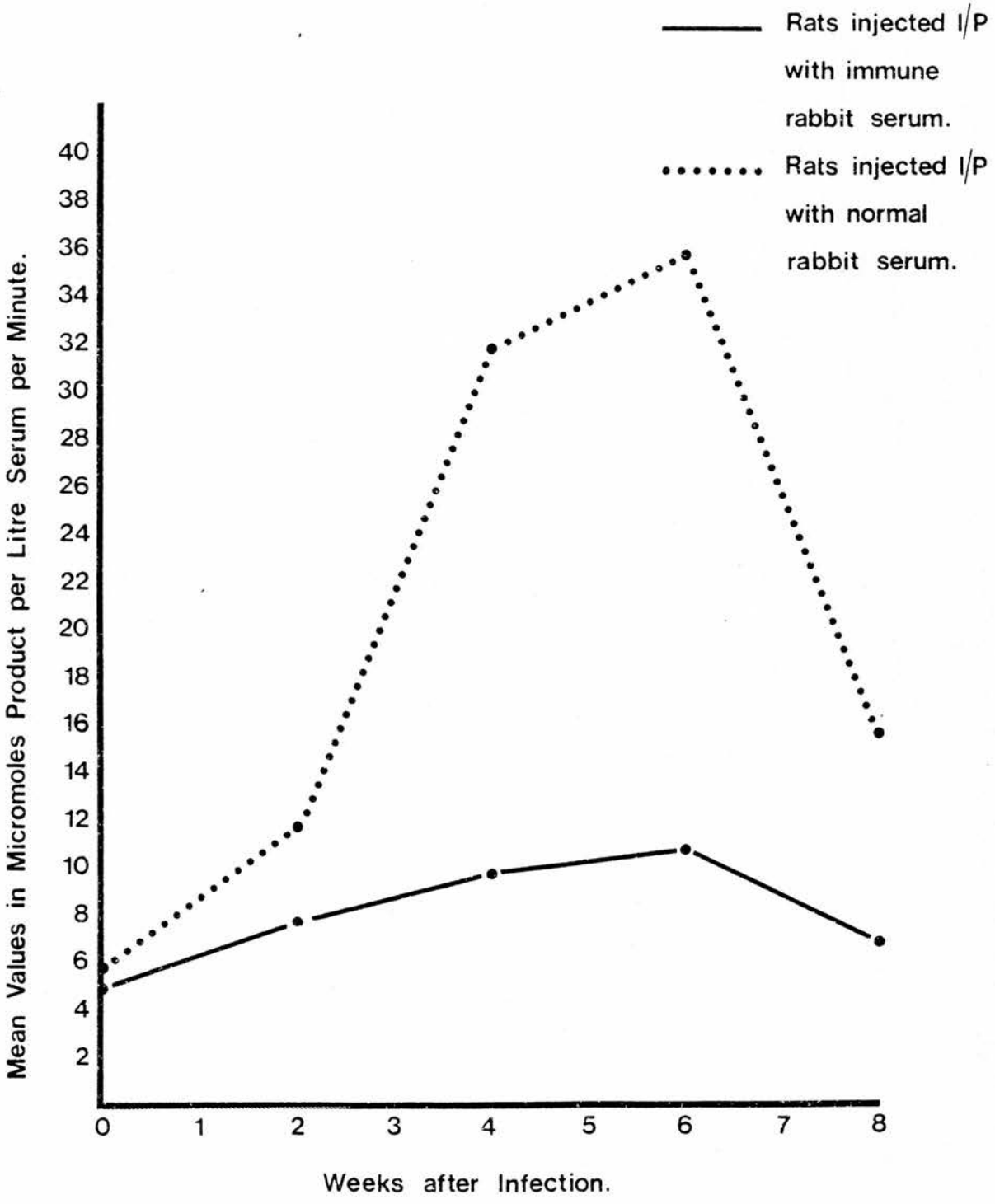


Fig.6·8.

The Effect of I/P Transfer of Immune Rabbit Serum on the Immunity to *F. hepatica* in Rats. Serum Glutamic Dehydrogenase Levels.



6.5. The effect of intraperitoneal transfer of immune bovine serum on resistance to F. hepatica in rats

Introduction

It has been suggested previously that infections with F. hepatica in rats and rabbits can be used as models for the similar infections in cattle and sheep respectively. The protective effect of immune rat and rabbit sera have been investigated and it therefore completes the comparisons to study the protective value of bovine and ovine immune sera. Furthermore, as much larger volumes of bovine or ovine sera can be obtained, as compared with those from rats or rabbits, these offer considerable advantages for further studies on the passive transfer system.

Experimental design

The donors of immune bovine serum were two calves each infected with 750 metacercariae. Serum was collected at the 8th, 9th and 10th weeks after infection. The calves were reinfected after 22 weeks and more serum was collected 8 weeks later. Precipitating antibodies were detected against somatic fluke antigen in every batch of serum collected.

Eighteen rats were divided into 2 groups, A (10 rats) and B (8 rats) and each rat was infected with 20 metacercariae. Immediately after infection the rats in group A were each injected intraperitoneally with 10 ml of immune bovine serum and this was repeated after 2 days. Rats in group B were similarly injected with normal bovine serum. Serum glutamic dehydrogenase activity was determined fortnightly and post-mortem examination was carried out 8 weeks after infection.

Results

Fluke recovery

This is recorded in Table 6.5. A highly significant difference was found between the numbers of flukes recovered from the two groups.

Serum glutamic dehydrogenase assays

The results are shown in Appendix Table 6.9. and Fig. 6.9. The enzyme level increased after infection in both groups reaching a peak at the 4th week in the rats injected with immune bovine serum but not until the 6th week in the rats injected with normal bovine serum. The enzyme activity in the latter rats was higher than that in the former at both the 4th and 6th weeks but the difference was significant only at the latter time ($t = 2.59$, $P < 0.02$).

Discussion

It is clear that immune bovine serum can confer resistance on recepient rats. This serum was therefore used in several subsequent passive-transfer experiments because large volumes can easily be obtained.

6.6. The effect of intraperitoneal transfer of immune ovine serum on the resistance to *F. hepatica* by rats

Introduction

This experiment was intended to complete the study on the protective effects of immune sera from the four host species.

Experimental design

Donors of immune ovine serum had been infected with 1000 metacercariae 12 weeks previously. Precipitating antibodies were detected in the sera against somatic fluke antigen.

TABLE 6.5.

The effect of intraperitoneal transfer of immune bovine serum on resistance to F. hepatica by rats.

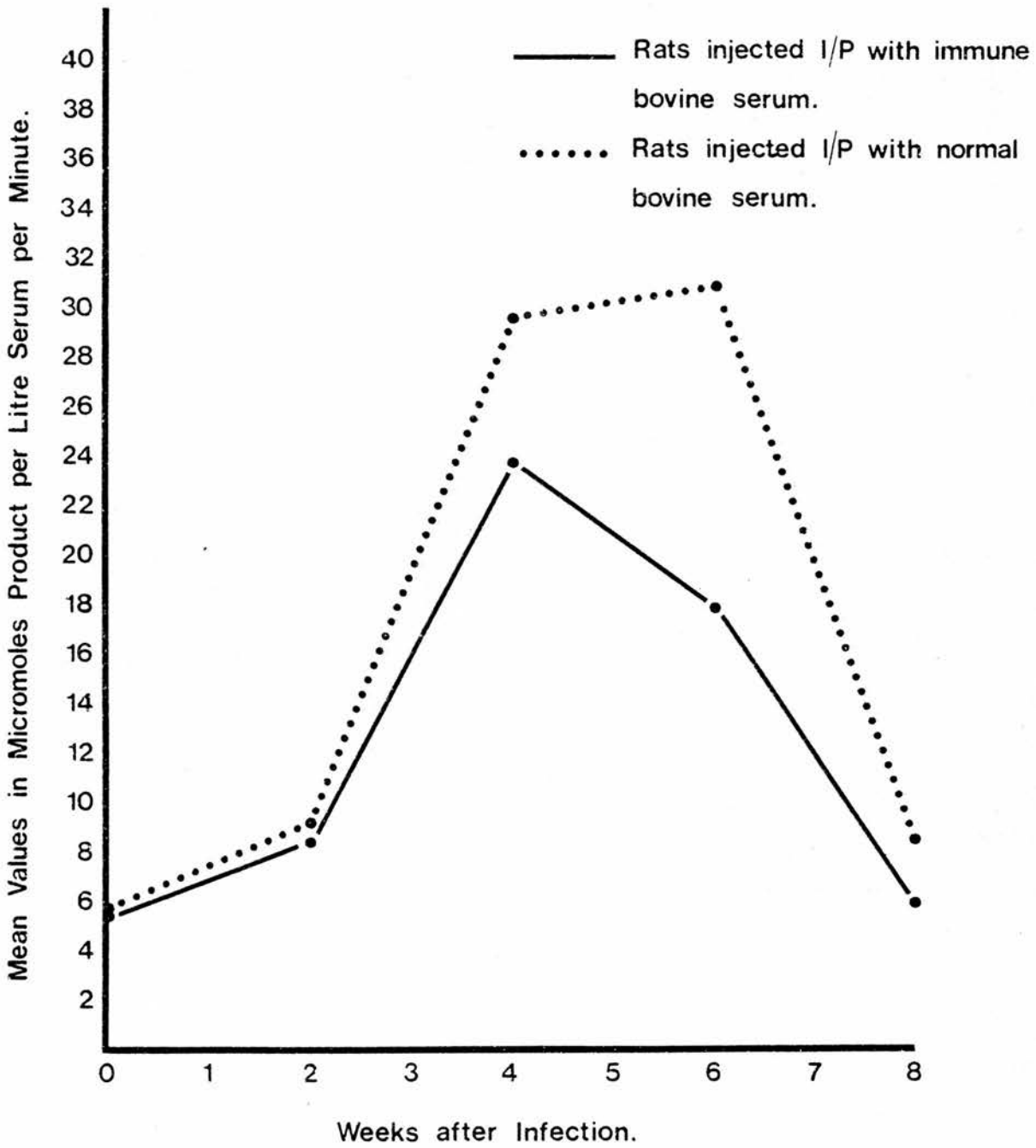
Rats group	challenge infection (metacercariae)	Nos. of flukes recovered at p.m.	
		Individual	mean ± S.D.
2 x 10 ml immune bovine serum I/P at days 0 and 2 (A)	20	0, 1, 1, 1, 1 2, 2, 2, 3, 4	1.7 ± 1.2
2 x 10 ml normal bovine serum I/P at days 0 and 2 (B)	20	2, 2, 2, 3, 4 5, 5, 6	3.6 ± 1.6

By Wilcoxon's two-sample test:

A v B, $P < 0.01$

Fig. 6·9.

The Effect of I/P Transfer of Immune Bovine Serum on the Resistance to F. hepatica by Rats. Serum Glutamic Dehydrogenase Levels.



Two groups (A and B) each of 5 rats were used. The rats were each infected with 20 metacercariae. Immediately after infection the rats in group A were each injected intra-peritoneally with 10 ml immune ovine serum and this was repeated after 2 days. Group B was similarly injected with normal ovine serum. Serum glutamic dehydrogenase activity was determined fortnightly and post-mortem examination was carried out after 8 weeks.

Results

Fluke recovery

This is recorded in Table 6.6. No significant difference was found between the numbers of flukes recovered from the two groups.

Serum glutamic dehydrogenase assays

The results are recorded in Appendix Table 6.10 and Fig. 6.10. The enzyme levels reached peaks at the 4th and 6th weeks in rats injected with normal ovine serum and immune ovine serum respectively. However, there was no significant difference between the enzyme levels in the two groups at any time.

Discussion

Immune ovine serum apparently failed to confer any protection on recepient rats although an equal volume of immune rabbit serum had previously been shown to be protective. This result was unexpected because of the previously mentioned similarities between rabbits and sheep. It was therefore decided to repeat this experiment with 7 rats in each group instead of 5.

TABLE 6.6.

The effect of intraperitoneal transfer of immune ovine serum on the resistance to F. hepatica by rats.

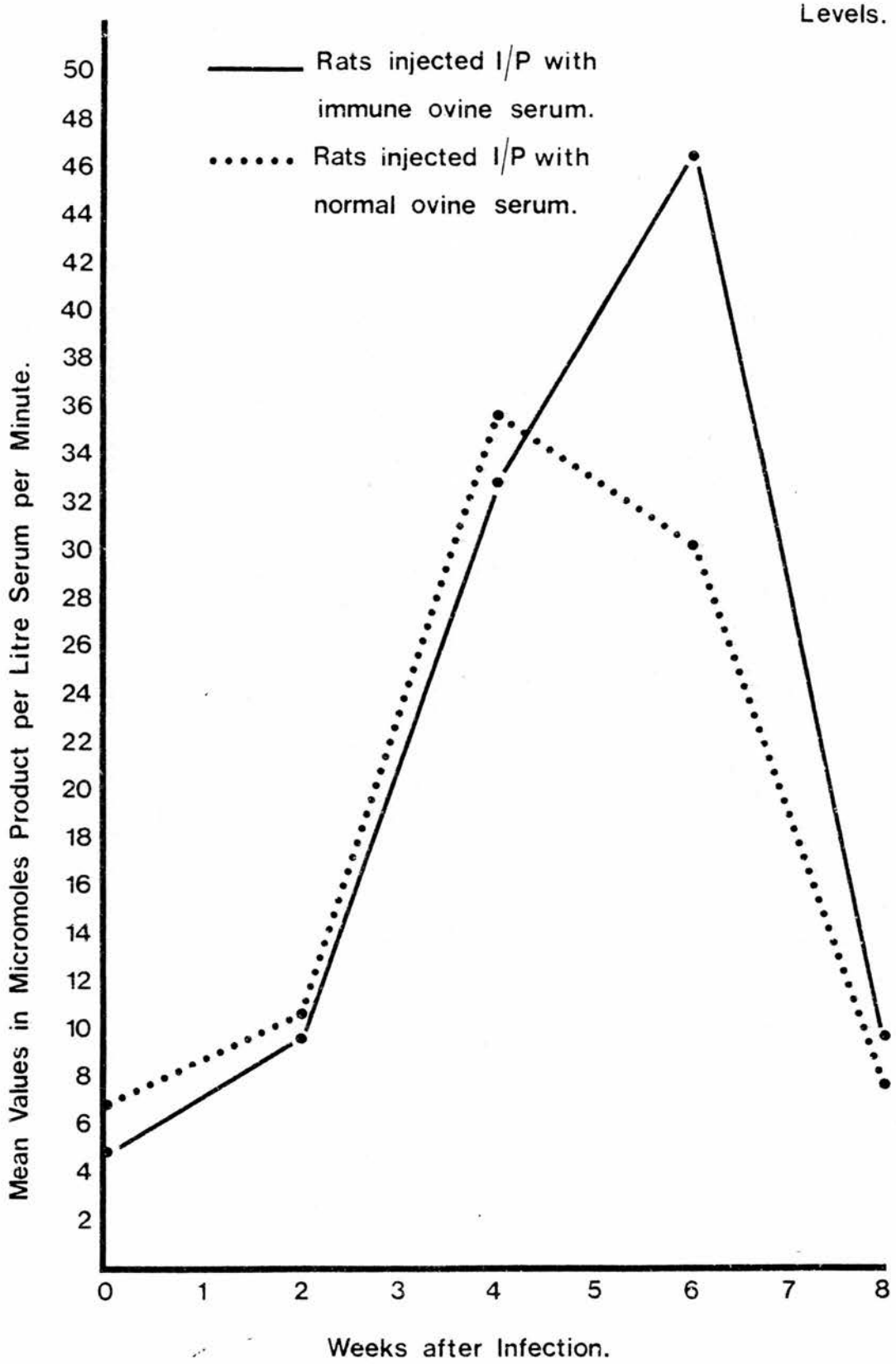
Rats group	challenge infection (metacercariae)	No. of flukes recovered at p.m.	
		Individual	mean ± S.D.
2 x 10 ml immune ovine serum I/P at days 0 and 2 (A)	20	1, 2, 4, 6, 7	4.0 ± 2.6
2 x 10 ml normal ovine serum I/P at days 0 and 2 (B)	20	1, 2, 3, 4, 6	3.2 ± 1.9

By Wilcoxon's two-sample test

A v B, $P > 0.05$, n.s.

Fig. 6·10.

The Effect of I/P Transfer of Immune Ovine Serum on the Immunity to F. hepatica in Rats. Expt. I. Serum Glutamic Dehydrogenase Levels.



6.7. Results

Fluke recovery

This is recorded in Table 6.7. Once again no significant difference was found between the numbers of flukes recovered from the two groups.

Serum glutamic dehydrogenase assays

The results are shown in Appendix Table 6.11. and Fig. 6.11. Once again the enzyme levels reached similar peaks at the 4th and 6th weeks in the rats injected with normal ovine serum and the rats injected with immune ovine serum respectively, and there was no significant difference between the mean enzyme activities in the two groups at any time.

Discussion

Again, no resistance was conferred on recepient rats by 10 ml of the immune ovine serum. However it may be that a larger volume of this serum would have confered protection or that the immune ovine serum had lost its protective effect by 12 weeks after infection. It is therefore not possible to conclude conclusively from these experiments that there is a distinct qualitative difference in the ability of rabbit and ovine sera from infected hosts to passively confer resistance to rats.

6.8. Passive transfer of resistance to *F. hepatica* in rats by gamma-globulin from immune homologous serum

Introduction

In this experiment the protective effect of gamma-globulin precipitated from an immune rat serum, which was itself known to be able to confer resistance on recepient rats, was

TABLE 6.7.

The effect of intraperitoneal transfer of immune ovine serum on the resistance to F. hepatica by rats.

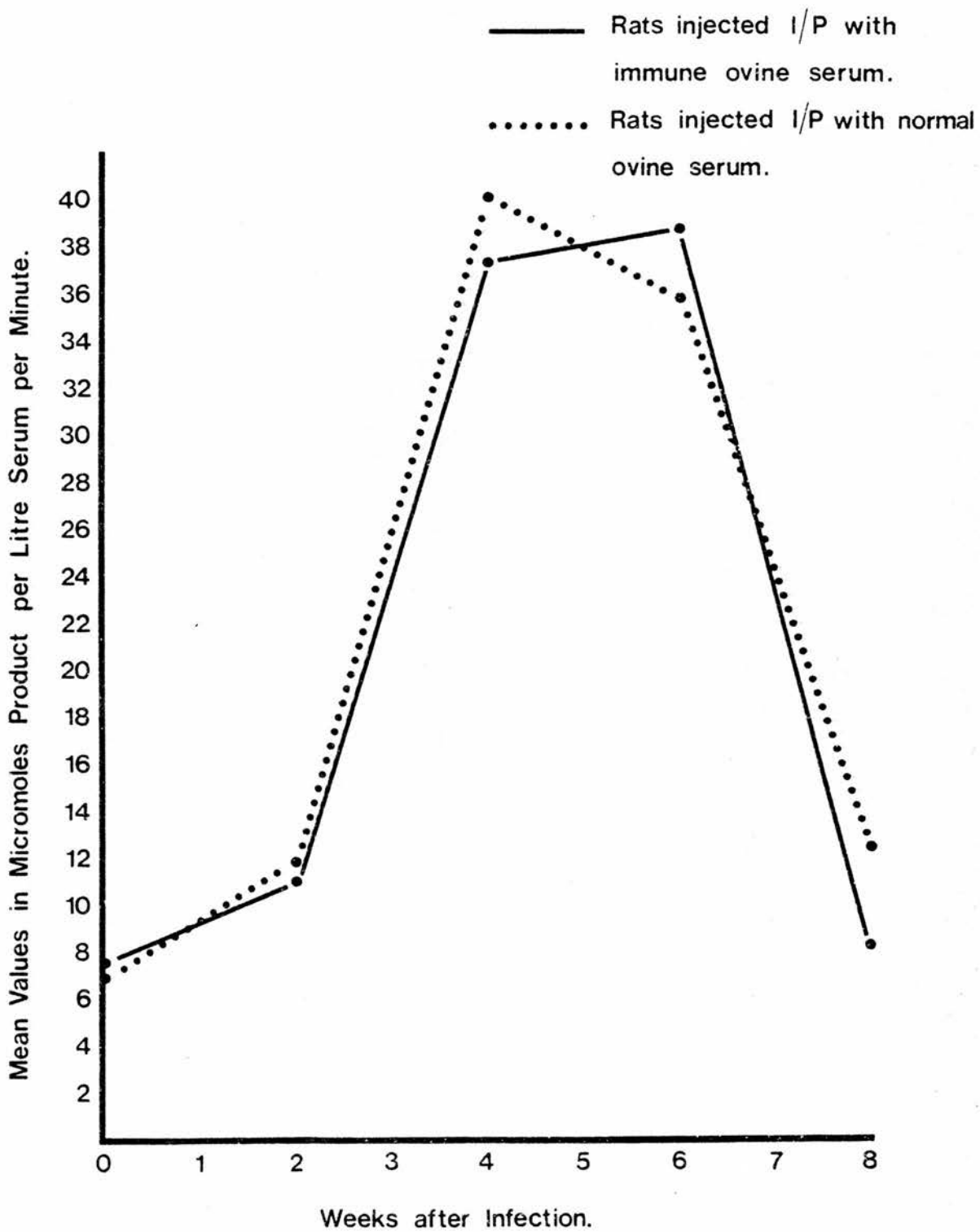
Rats group	challenge infection (metacercariae)	Nos. of flukes recovered at p.m.	
		Individual	mean ± S.D.
2 x 10 ml immune ovine serum I/P at days 0 and 2 (A)	20	2, 3, 3, 3, 4, 5, 9	4.1 ± 2.3
2 x 10 ml normal ovine serum I/P at days 0 and 2 (B)	20	3, 3, 3, 3 4, 4, 5	3.6 ± 0.8

By Wilcoxon's two-sample test:

A v B, $P > 0.05$, n.s.

Fig.6·11.

The Effect of I/P Transfer of Immune Ovine Serum on the Immunity to F.hepatica in Rats. Expt. II. Serum Glutamic Dehydrogenase Levels.



investigated. This was envisaged as the first step in determining the immunoglobulin class(es) responsible for this resistance and also as a useful step in facilitating absorbtion studies.

Experimental design

Twentythree rats were divided into 4 groups, A (8 rats), B (5 rats), C (5 rats) and D (5 rats). The rats were each injected with 20 metacercariae. Immediately after infection each rat in group A was injected intraperitoneally with 10 ml of precipitated gamma-globulin from an immune serum pool obtained from 30 rats infected with 20 metacercariae for 8 weeks and this was repeated after 2 days. Group B was similarly injected with gamma-globulin from normal rat serum. As a control for the protective value of the pool of immune serum from which the gamma-globulin had been precipitated, the rats in group C were each injected intraperitoneally with 8 ml of the original serum immediately after infection and again 2 days later. The rats in group B were similarly injected with normal rat serum. Serum glutamic dehydrogenase activity was determined fortnightly and post-mortem examination was carried out 8 weeks after infection.

Fluke recovery

This is recorded in Table 6.8. A significant difference was found in the numbers of flukes recovered from rats injected with immune gamma-globulin as compared with those from rats injected with normal gamma-globulin. A significant difference was also found between the numbers of flukes recovered from

TABLE 6.8.

Passive transfer of resistance to F. hepatica in rats by gamma-globulin from immune rat serum.

Rats group	challenge infection (metacercariae)	Nos. of flukes recovered at p.m.	
		Individual	mean ± S.D.
2 x 10 ml gamma-globulin from immune rat serum I/P on days 0 and 2 (A)	20	0, 0, 1, 2, 2, 3, 3, 3,	1.8 ± 1.3
2 x 10 ml gamma-globulin from normal rat serum I/P on days 0 and 2 (B)	20	3, 3, 3, 4, 5	3.6 ± 0.9
2 x 8 ml immune rat serum I/P at days 0 and 2 (C)	20	0, 1, 1, 1, 1	0.8 ± 0.4
2 x 8 ml normal rat serum I/P at days 0 and 2 (D)	20	3, 3, 3, 4, 6	3.8 ± 1.3

By Wilcoxon's two-sample test:

A v B, $P < 0.025$ A v C, $P > 0.05$, n.s.

C v D, $P < 0.005$ B v D, $P > 0.05$, n.s.

rats injected with immune rat serum and those from rats injected with normal rat serum. However, a comparison between the numbers of flukes recovered from rats injected with immune gamma-globulin and those from rats injected with immune rat serum showed no significant difference.

Serum glutamic dehydrogenase assays

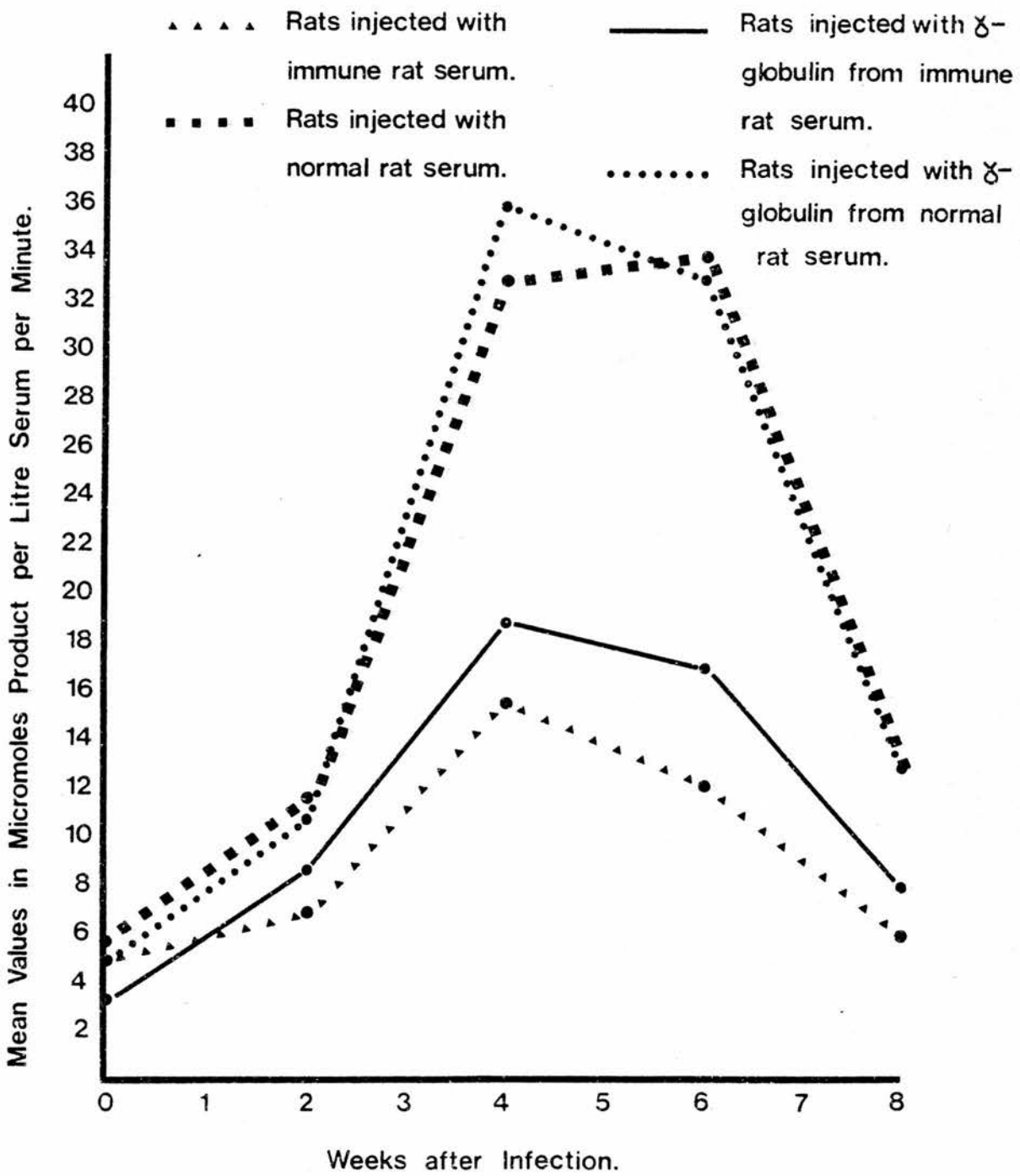
The results are recorded in Appendix Table 6.12. and Fig. 6.12. The enzyme level reached a peak at the 4th week in three of the groups and at the sixth week in the group given normal rat serum. However the elevation of the enzyme levels in the rats injected with immune gamma-globulin or immune rat serum was reduced. The difference between the enzyme activities of the rats given the immune gamma-globulin and those injected with normal gamma-globulin was found to be significant at both the 4th ($t = 2.41$, $P < 0.05$) and the 6th ($t = 2.59$, $P < 0.05$) weeks. Similarly, a significant difference was found in the mean enzyme activity of the rats injected with immune rat serum as compared with that of rats injected with normal rat serum at the 4th ($t = 2.96$, $P < 0.02$) and 6th ($t = 3.08$, $P < 0.02$) weeks. No significant differences were found at any time in the mean enzyme activities of the rats injected with immune gamma-globulin as compared with rats injected with immune serum or between the rats given normal gamma-globulin as compared with those given normal serum at any time.

6.9. Passive transfer of resistance to *F. hepatica* in rats by gamma-globulin from immune bovine serum

Introduction

In this experiment the protective effect of gamma-

Fig.6·12. Serum Glutamic Dehydrogenase Levels.



globulin precipitated from the same pool of immune bovine serum as was used in experiment 6.5. was investigated.

Experimental design

A similar design to the previous experiment was adopted using gamma-globulin from normal and immune bovine serum in two groups (A and B) each containing 6 rats.

Results

Fluke recovery

This is recorded in Table 6.9. A significant difference was found between the numbers of flukes recovered from the rats injected with immune bovine gamma-globulin and that from the rats injected with normal bovine gamma-globulin.

Serum glutamic dehydrogenase assays

The results are shown in Appendix Table 6.13. and Fig. 6.13. In both groups the enzyme levels reached a peak at the 4th week. However, the peak was significantly lower ($t = 2.39$, $P < 0.05$) in the rats injected with immune bovine gamma-globulin as was the level 6 weeks after infection ($t = 3.27$, $P < 0.01$).

Discussion

The results of this and the previous experiment clearly show that both immune bovine and rat gamma-globulin can confer resistance to recepient rats. The next step in this study would be to fractionate the gamma-globulin to determine the immunoglobulin class(es) involved in the resistance.

6.10. Passive transfer of resistance to *F. hepatica* in rabbits by concentrated immune bovine serum

It had been intended to investigate the protective effect

TABLE 6.9.

Passive transfer of resistance to F. hepatica in rats by gamma-globulin from immune bovine serum.

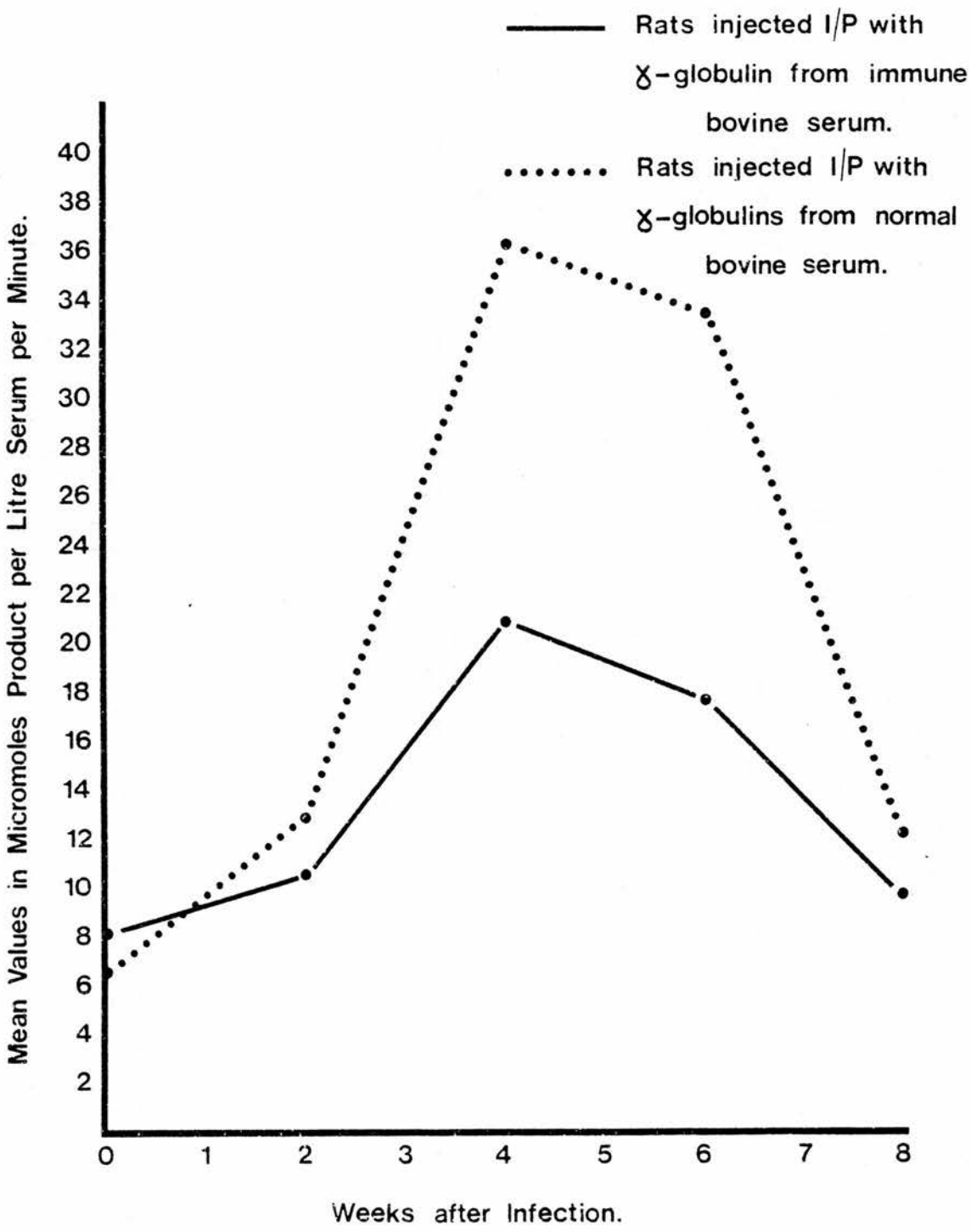
Rats group	challenge infection (metacercariae)	Nos. of flukes recovered at p.m.	
		Individual	mean ± S.D.
2 x 10 ml gamma-globulin from immune bovine serum I/P at days 0 and 2 (A)	20	0, 0, 1 2, 2, 3	1.3 ± 1.2
2 x 10 ml gamma-globulin from normal bovine serum I/P at days 0 and 2 (B)	20	2, 3, 3, 3, 4, 5	3.3 ± 1.0

By Wilcoxon's two-sample test:

A v B, $P \leq 0.025$

Fig.6·13.

Passive Transfer of Immunity to F.hepatica in Rats by γ-globulin from Immune Bovine Serum. Serum Glutamic Dehyhdrogenase Levels.



of immune bovine serum in rabbits using a lower ratio of serum volume to recepient weight as in rats (i.e. less than 1:15). Normal and immune bovine sera were therefore concentrated to one third of the original volume. However it was found that about 20 ml of such serum was the maximum volume that could be given without risking the rabbits dying of serum sickness. Accordingly, only a pilot study was carried out to determine the efficacy of this concentrated serum in passively transferring resistance.

Experimental design

Two groups (A and B) each comprising 3 rabbits were used and each rabbit was injected with 50 metacercariae. Immediately after infection the rabbits in group A were each injected with 10 ml of concentrated immune bovine serum and this was repeated after 2 days. Group B was similarly injected with concentrated normal bovine serum. All the rabbits were necropsied 8 weeks after infection.

Results

Fluke recovery

This is recorded in Table 6.10. A significant difference was found between the numbers of flukes recovered from the two groups.

Discussion

A volume of 20 ml of concentrated immune bovine serum (equivalent to 60 ml unconcentrated serum) conferred a significant resistance in rabbits. The fact that a similar effective volume (50 ml) of unconcentrated immune rat serum also conferred significant protection in rabbits whereas a

TABLE 6.10.

Passive transfer of resistance to F. hepatica in rabbits by concentrated immune bovine serum.

Rabbit group	challenge infection (metacercariae)	Nos. of flukes recovered at p.m.	
		Individual	mean ± S.D.
2 x 10 ml concentrated immune bovine serum at days 0 and 2 I/P (A)	50	4, 5, 5,	4.67 ± 0.58
2 x 10 ml concentrated normal bovine serum at days 0 and 2 I/P (B)	50	9, 10, 12	10.33 ± 1.53

By Wilcoxon's two-sample test:

A v B, $P < 0.05$

similar volume of immune rabbit serum failed to do so indicates that the immune rat and bovine sera have a higher protective value against F. hepatica than immune rabbit serum. It was unfortunately impossible in the time available to repeat the experiment with a larger number of rabbits.

6.11. Passive transfer of resistance to F. hepatica in rats by serum from rats implanted with mature flukes encapsulated in diffusion chambers

Experimental design

Fifteen donor rats were implanted intraperitoneally with adult flukes encapsulated in filtration-membrane diffusion chambers and serum was collected after 2 weeks. Precipitating antibodies were detected against somatic fluke antigen.

Fourteen rats were divided into two equal groups (A and B). All the rats were infected with 20 metacercariae. Immediately after infection, each rat in group A was injected intraperitoneally with 7.5 ml of serum obtained from rats implanted with flukes in diffusion chambers and this was repeated after 2 days. Group B was similarly injected with serum from rats implanted with empty chambers. Serum glutamic dehydrogenase activities were determined fortnightly and post-mortem examination was carried out 8 weeks after infection.

Results

Fluke recovery

This is recorded in Table 6.11. A significant difference was found between the numbers of flukes recovered from the two groups.

TABLE 6.11.

Passive transfer of resistance to F. hepatica in rats by serum from rats implanted with mature flukes encapsulated in diffusion chambers.

Rats group	challenge infection (metacercariae)	Nos. of flukes recovered at p.m.	
		Individual	mean ± S.D.
2 x 7.5 ml serum from rats implanted with mature flukes in chambers at days 0 and 2 I/P (A)	20	0, 1, 2, 2, 3, 3, 3	2.0 ± 1.2
2 x 7.5 ml serum from rats implanted with empty chambers at days 0 and 2 I/P (B)	20	1, 2, 4, 4 6, 6, 6	4.1 ± 2.0

By Wilcoxon's two-sample test:

A v B, $P < 0.05$

Serum glutamic dehydrogenase assays

The results are shown in Appendix Table 6.14. and Fig. 6.14. The enzyme levels in both groups reached a peak at the 4th week. However, the peak was significantly lower in the group injected with serum from rats implanted with encapsulated flukes ($t = 2.49$, $P < 0.05$).

Discussion

It has previously been shown that the metabolic products which diffuse through filtration membrane diffusion chambers containing mature flukes are antigenic and can stimulate resistance to challenge. It is now clear that some humoral agent(s) which are thus produced can passively transfer this resistance.

6.12.1. Absorption of the protective component(s) from immune bovine serum

Introduction

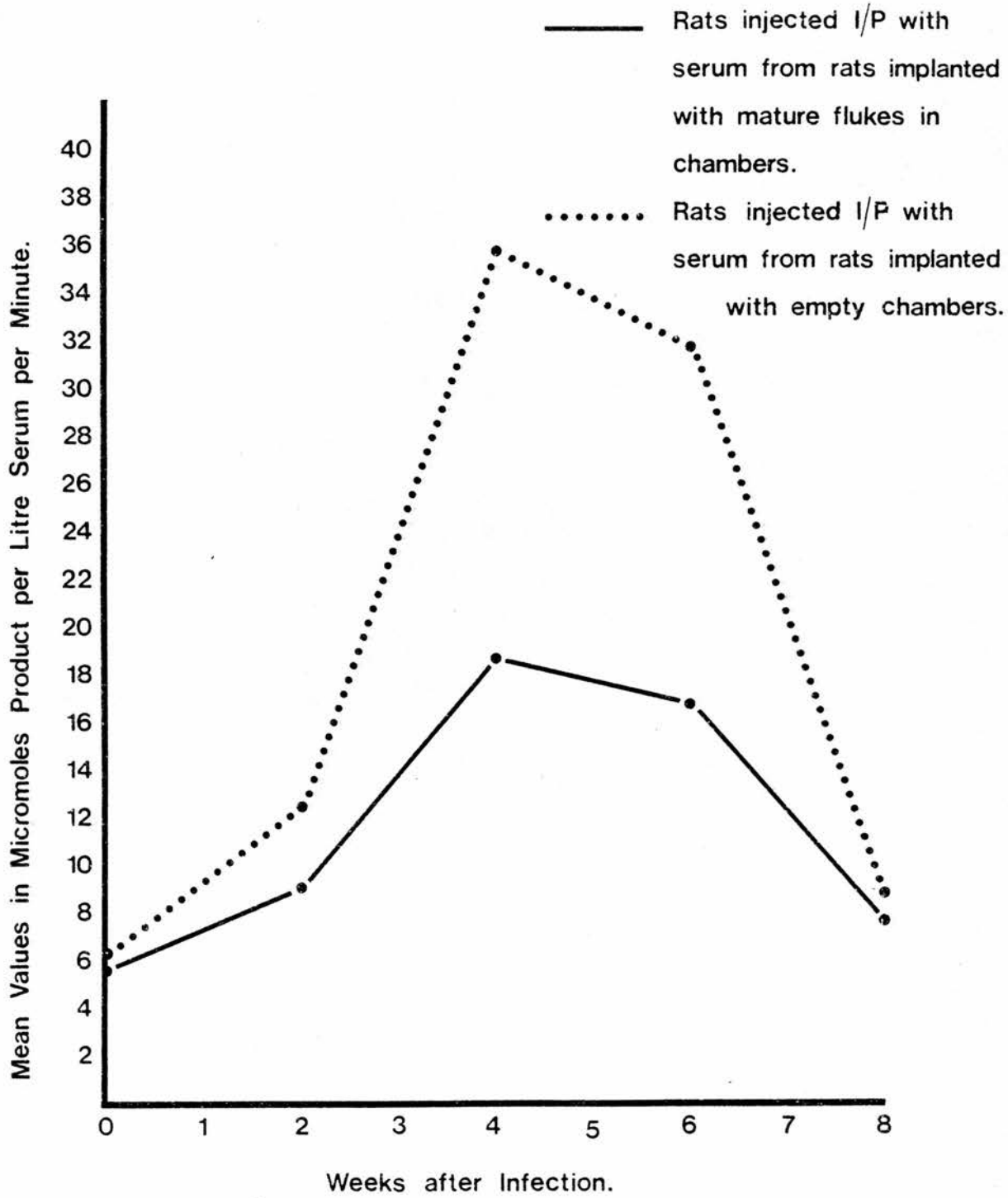
Attempts were made to determine whether the protective component in immune serum can be absorbed by direct contact with mature flukes or by metabolic products diffusing from mature flukes encapsulated in chambers.

Experimental design

Twentythree rats were divided into three groups A (8 rats), B (8 rats) and C (7 rats). Each rat was infected with 20 metacercariae. Immediately after infection rats in group A were each injected intraperitoneally with 10 ml of immune bovine serum in which mature flukes had been maintained for 48 hours at 37°C and this was repeated after 2 days. Group B was similarly injected with immune bovine serum in which

Fig. 6·14.

Passive Transfer of Resistance to F. hepatica in Rats by Serum from Rats Implanted with Mature Flukes Encapsulated in Diffusion Chambers. Serum Glutamic Dehydrogenase Levels.



mature flukes encapsulated in diffusion chambers had been maintained for 48 hours at 37°C and group C with normal bovine serum. Serum glutamic dehydrogenase activity was determined fortnightly and post-mortem examination was carried out 8 weeks after infection.

Results

Serology

Both absorbed sera still showed precipitation lines against somatic fluke antigen in agar.

Fluke recovery

This is recorded in Table 6.12. There were no significant differences between the numbers of flukes recovered from any of the groups.

Serum glutamic dehydrogenase assays

Results are shown in Appendix Table 6.15. and Fig. 6.15. Again there were no significant differences between the mean enzyme levels in any of the groups at any time, all reaching a peak at the 4th week.

Discussion

This experiment appeared to show that directly or indirectly absorbed immune serum lost its protective effect. However, as there was no group of rats injected with un-absorbed immune serum kept at 37°C from 48 hours it is possible that the loss of protection was simply due to this incubation at an elevated temperature. It was therefore decided to repeat this experiment in a modified form.

I: Absorption of the protective component from immune bovine serum.

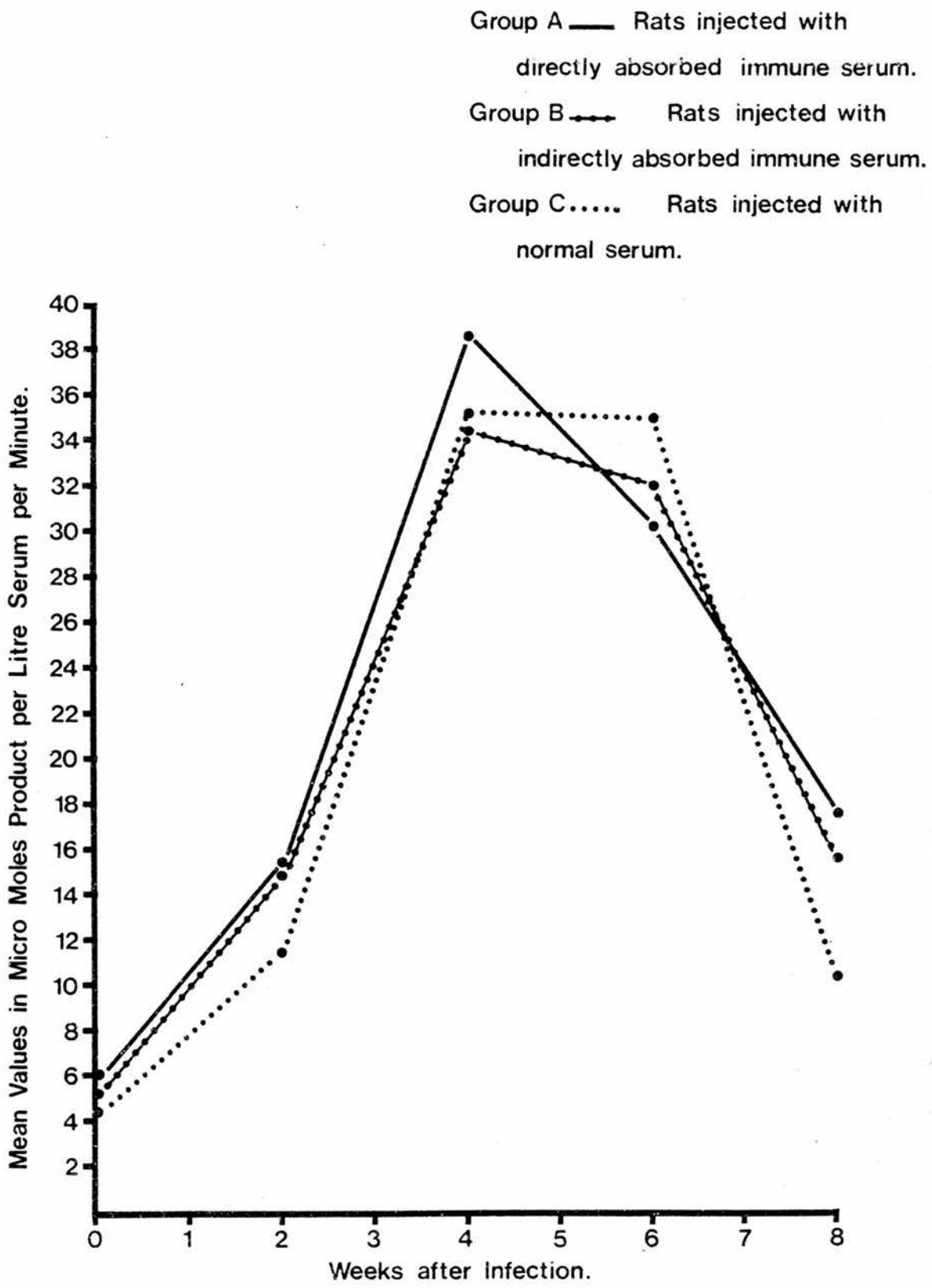
Rats group	challenge infection (metacercariae)	Nos. of flukes recovered at p.m.	
		Individual	mean $\pm$ S.D.
2 x 10 ml immune bovine serum in which mature flukes had been maintained at days 0 and 2 I/P (A)	20	3, 3, 3, 4, 5, 6, 7, 8	4.9 $\pm$ 1.96
2 x 10 ml immune bovine serum in which encapsulated mature flukes had been maintained at days 0 and 2 I/P (B)	20	2, 3, 3, 4, 6 6, 6, 6	4.5 $\pm$ 1.69
2 x 10 ml normal bovine serum at days 0 and 2 I/P (C)	20	2, 2, 2, 3, 3, 4, 6	3.14 $\pm$ 1.46

By Wilcoxon's two sample test:

A and C, $P > 0.05$, n.s.

B and C, $P > 0.05$, n.s.

Fig.6·15. Serum Glutamic Dehydrogenase Levels.



6.12.2. Absorption of the protective components from immune bovine serum

Experimental design

Eighteen rats were divided into 3 groups A (7 rats), B (4 rats) and C (7 rats). Each rat was infected with 20 metacercariae. Immediately after infection the rats in group A were each injected intraperitoneally with 10 ml of immune bovine serum which had been kept at 37°C for 48 hours and this was repeated after 2 days. Groups B and C were similarly injected with normal bovine serum and immune bovine serum absorbed by direct contact with living flukes for 48 hours respectively. All rats were necropsied 8 weeks after infection.

Results

Serology

The absorbed serum still showed precipitation lines against somatic fluke antigen.

Fluke recovery

This is recorded in Table 6.13. A significant difference was found between the numbers of flukes recovered from the group injected with immune bovine serum kept at 37°C for 48 hours and that from the group injected with normal bovine serum. However, although there were 32% fewer flukes recovered from the group injected with immune bovine serum kept at 37°C for 48 hours when compared with that from the group injected with directly absorbed serum, this difference was not statistically significant.

Discussion

The results of this experiment are again inconclusive, although they tend to support the suggestion from the previous

TABLE 6.13.

II: Absorption of the protective component from immune bovine serum.

Rats group	challenge infection (metacercariae)	Nos. of flukes recovered at p.m.	
		Individual	mean ± S.D.
2 x 10 ml immune bovine serum kept at 37°C for 48 hours at days 0 and 2 I/P (A)	20	1, 1, 2, 2, 3 5, 5	2.71 ± 1.71
2 x 10 ml normal bovine serum at days 0 and 2 I/P (B)	20	4, 4, 6, 6	5.00 ± 1.15
2 x 10 ml immune bovine serum in which mature flukes were maintained for 48 hours at days 0 and 2 I/P (C)	20	2, 2, 3, 4, 4, 4, 9	4.00 ± 2.38

By Wilcoxon's two-sample test:

A v B, $P < 0.05$

B v C, $P > 0.05$, n.s.

A v C, $P > 0.05$, n.s.

experiment that the protective components of immune serum can be absorbed out by the presence of living flukes. It appears probable that either the absorbtion was incomplete or that keeping serum at 37°C for 48 hours reduces its protective ability. Indeed both factors may be involved. This study was not repeated again partly because there was insufficient time available but also because its importance was overtaken by events in the form of the results from other concurrent absorption experiments.

6.12.3. Absorption of the protective factor(s) from gamma-globulin precipitated from infected bovine serum

Introduction

In this experiment gamma-globulins precipitated from infected bovine serum with ammonium sulphate were absorbed with the volume of metabolic products from adult flukes maintained *in vitro* sufficient to just remove all immuno-precipitating activity. These absorbed globulins were then injected into rats to see whether they had lost their protective effect.

Experimental design

Eighteen rats were divided into 3 equal groups A, B and C. Each rat was injected with 20 metacercariae. Immediately after infection the rats in group A were each injected intraperitoneally with 6 ml of gamma-globulin from infected bovine serum and this was repeated 2 days later. Group B was similarly injected with gamma-globulin from normal bovine serum and group C with the absorbed gamma-globulin from immune bovine serum.

Results

Fluke recovery

This is recorded in Table 6.14. There were significant differences between the numbers of flukes recovered from the rats injected with gamma-globulin from immune bovine serum as compared with those from the rats injected with either gamma-globulin from normal bovine serum or absorbed gamma-globulin from immune bovine serum. Furthermore, no significant difference was found between the numbers of flukes recovered from the rats in the latter two groups.

Discussion

It is clear that the absorbed immune gamma-globulin failed to confer resistance to recepient rats. Hence it appears that the protective 'antibodies' were inactivated by some component(s) in the metabolic products obtained from the cultured flukes. Lehner (1977) showed that such metabolic products collected from flukes maintained *in vitro* do not induce an active resistance when used as a vaccine. It therefore seems possible that such metabolic products contain a hapten which reacts with and inactivates the protective antibody.

The results of this experiment also suggested that the tentative but not conventionally significant results of the previous two experiments are in fact valid.

III: Absorption of the protective component from gamma-globulin precipitated from immune bovine serum.

Rats group	challenge infection (metacercariae)	Nos. of flukes recovered at p.m.	
		Individual	mean ± S.D.
2 x 6 ml gamma-globulin from immune bovine serum at days 0 and 2 I/P (A)	20	0, 1, 2, 2, 2, 4	1.83 ± 1.33
2 x 6 ml gamma-globulin from normal bovine serum at days 0 and 2 I/P (B)	20	2, 2, 4, 4, 4, 5	3.5 ± 1.22
2 x 6 ml absorbed gamma-globulin from immune bovine serum at days 0 and 2 I/P (C)	20	3, 3, 4, 4, 4, 6	4.00 ± 1.10

By Wilcoxon's two-sample test:

A v B, $P < 0.05$

A v C, $P < 0.025$

B v C, $P > 0.05$, n.s.

CHAPTER 7

GENERAL DISCUSSION

It is clear that resistance to F. hepatica can be actively stimulated in rats by immature or mature previous infections which have been eliminated by anthelmintics. The involvement of immunological factors in such resistance has been confirmed by the finding that metabolic products diffusing from mature flukes encapsulated in diffusion chambers, which had been implanted subcutaneously or intraperitoneally in rats, can stimulate a protective response. These findings support previous reports by Eriksen and Flagstad (1974b) and Anderson, Hughes and Harness (1975). The former authors reported that implantation of mature flukes subcutaneously in rats for 4 weeks resulted in a 50% decrease in the numbers of flukes recovered from oral challenge as compared to non-implanted controls. Anderson et al. (1975) also reported that histological examination of the prefemoral lymph nodes of rats implanted subcutaneously with one adult fluke for 12 days showed evidence of cell-mediated immunity and concomittant production of antibody. Furthermore, there was intradermal hypersensitivity to fluke antigen and precipitating antibodies were formed.

The fact that diffusible antigens, from encapsulated mature flukes which had been implanted in rats, stimulated a protective immunological response excludes any essential immunogenic role for the flukes' tegumental cells or eggs since these are too large to pass through the diffusion

chambers with a pore size of 0.45 um.

On the other hand, the present findings conflict with reports by Goose (1977) and Rajasekariah and Howell (1978). The former author reported that mature flukes implanted intraperitoneally in rats completely failed to stimulate resistance to oral challenge. However, it is possible that this failure was due to the fact that he challenged his rats 13 weeks after implantation, by which time the implanted flukes were dead.

Rajasekariah and Howell (1978) however reported the failure of subcutaneous implantation of mature flukes to stimulate resistance to oral challenge in rats 2 or 4 weeks after implantation. However they found that 4-week-old flukes, metacercariae or fluke eggs would all stimulate a significant resistance to challenge 2 weeks after implantation. The implanted metacercariae reached the liver where they caused damage. However no hepatic damage was found in rats implanted with eggs or 4-week-old flukes. The authors suggested that implanted eggs were disseminated more widely than eggs produced by implanted mature flukes because the latter were encapsulated within a nodule, while the former "may be processed more effectively by the defence mechanisms of the host". They thus suggested that such immature eggs and the/flukes are more immunogenic than adult flukes. However, implanted adult flukes would be expected to produce a lot of eggs before they were encysted. Therefore, a more likely explanation is perhaps the alternative one

given by the same authors, that the discrepency between their results and those of other authors about the immunogenicity of implanted mature flukes may be attributed to differences in the strains of rats in the different experiments.

While implantation of mature flukes usually stimulates significant resistance to F. hepatica in rats, it fails to do so in rabbits. Hughes and Harness (1973a) reported that mature flukes implanted into rats were found from the third day within cysts in the body cavity, liver or the scrotal sac. It appears that the short period before cyst formation is enough for the implanted rats to develop a protective immunological response against F. hepatica, or that the antigens can diffuse from such cysts in rats.

In rabbits it was found in the present work that the flukes quickly die after subcutaneous implantation and do not become encysted so that the quantity of antigenic stimulus may not be sufficient to stimulate a protective immunological response. Similarly Hughes and Harness (1972; 1973a) and Eriksen and Flagstad (1974b) reported that adult flukes implanted into rabbits body cavities do not satisfactorily adapt and thrive, whereas in rats implanted mature flukes can live within cysts up to 12 weeks, whereas no cysts were formed around dead flukes or flukes which died shortly after transfer. It is also possible that rabbits are incapable of recognising and responding to those 'essential' antigens which can stimulate a protective immune response, as suggested by Hughes (1963).

The involvement of immunological response in the resistance of F. hepatica was also demonstrated in the present work by passive transfer of a humoral agent contained within the gamma-globulin fraction of serum and hence probably an antibody. However, the successful transfer of such resistance appears to depend on many factors such as the volume of immune serum transferred, the time of transfer, the duration of the infection in the donor and the species of the recepient and donor. Thus immune rat and bovine sera as well as gamma-globulin precipitated from such sera were found to protect recepient rats and rabbits against challenge with F. hepatica. However immune rabbit serum was found not to confer resistance on recepient rabbits although, perhaps surprisingly it was protective to rats. This difference may have been due to the larger ratio of serum to recepient liveweight in the rats, but sheep serum failed to confer any resistance on recepient rats in a volume similar to that at which rat or bovine sera were effective.

The importance of the volume and time of immune serum transfer was first shown by Armour and Dargie (1974), who found a direct relationship between the volume of serum transferred and the degree of resistance obtained. Thus the mean fluke recoveries from a challenge infection of 20 metacercariae in rats which had received 0, 5, 10 or 20 ml were 3.6, 2, 1.4 and 0.6 respectively and successful protection was obtained only when sera was given at the time of challenge. On the other hand, Hayes, Bailer and Mitrovic

(1974b) found no significant difference in protective effect between 5, 2.5 and 1 ml of immune homologous serum transferred into rats. However, these authors also emphasised the importance of the time of serum transfer as immune serum given at the day of infection was protective, whereas when it was given 2 or 4 days after infection there was only a slight protective effect and when it was given 6 or 8 days after challenge there was no effect. Such factors as an insufficient volume of serum transferred into recepients or incorrect time of transfer may have been responsible for the failure of earlier attempts to transfer immunity passively (Wikerhauser, 1961b; Corba, Armour, Roberts and Urquhart, 1971).

In the present work immune serum was collected from different donor species after about 8-weeks of infection except from sheep which had 12-week-old infections. It was not expected that this would affect the protective capacity of the serum. However, an analogous situation in rats suggests that this may be so. Howell, Sandeman and Rajasekariah (1977) reported that serum from donor rats with 13-20 week-old infections was protective to recepients whereas Hayes, Bailer and Mitrovic (1974b)found that serum from rats with a 25-week-old infection was not protective. This might be related to the decrease in the antibody titre in older infections although it should be pointed out that high serological titres are not necessarily associated with resistance, as was found in the present work in rabbits and sheep. Alternatively, Van Tiggele (1975) proposed that since sheep are more efficient

producers of antibody to antigenic stimulus than calves, the former may produce excessive amounts of blocking antibodies, with a consequent poor immunity in terms of resistance.

It is therefore clear that humoral immunity can be involved in the resistance to F. hepatica. This was further confirmed by the finding that the protective effect can be absorbed out of both immune serum and immune gamma-globulin. However, as has been mentioned in chapter 3, resistance can also be passively transferred by lymphocytes. Dargie, Armour, Rushton and Murray (1974) suggested that either these lymphocytes are attracted to the sites of antigen deposition and stimulate a cell-mediated reaction or that they themselves produce antibodies against the flukes in the recepient. Since rats protected by immune serum transfer usually have normal livers while those protected by lymphocytes show evidence of hepatic damage, cellular infiltrates and dead immature flukes, these authors suggested that in the former case only, the young flukes are killed prior to entry into the liver, while both types of immunity are involved in natural resistance to F. hepatica, the cellular response acting as a second line of defence.

There are, however, several other hypotheses which attempt to explain the mechanism of resistance to F. hepatica. These are mainly divided into two major groups. One of these includes views which consider non-specific factors such as competitive inhibition in high level infections or environmental

changes in the liver due to inflammation and fibrosis caused by primary infections to be responsible for the resistance against F. hepatica in repeatedly infected hosts. (Thorpe, 1965; Bolbol, 1975; Hughes, Harness and Doy, 1977a).

Such non-specific factors may account for the reduction in the number of flukes recovered from rabbits with repeated infections, (Bolbol, 1975) but, there is undoubtedly a specific immunological mechanism in rats. Thus, in spite of the fact that liver damage and fibrosis in the rats which had experienced two immature infections and treatments appeared greater than in the rats challenged after treatment of a single mature infection, the level of resistance was similar in the two groups. Hence, in rats, resistance to F. hepatica does not seem to be directly related to the degree of hepatic fibrosis. It would have been desirable to confirm this point by histopathological evidence as well. However, these findings are in agreement with Hayes, Bailer and Mitrovic (1973) who reported that a primary infection with one fluke was sufficient to confer a high degree of resistance to challenge after 7 weeks in rats, although such livers showed little damage at the time of challenge. They also showed (1974a) that long-standing infections, of 7 or 12 months duration, conferred a significant degree of resistance to reinfection, even though regenerative and healing processes had occurred. The present work has also clearly shown that metabolic products from mature flukes stimulate significant resistance in rats and this further refutes the

view that non-specific inflammatory and fibrotic changes are exclusively responsible for the resistance of repeatedly infected rats.

Another group of hypotheses consider a pre-hepatic or hepatic immune response associated with immature fluke antigens to be responsible for the resistance in repeatedly infected animals. Thus, Hayes and Mitrovic (1977), Rajasekariah and Howell (1977b) and Doy, Hughes and Harness (1978) maintained that the protective immunological response occurs in the intestinal wall in rats; and Fortmeyer (1974) suggested that a similar mechanism occurs in rabbits. Doy et al. suggested that the invading immature flukes from the challenge infection trigger off a local anaphylactic reaction as they migrate through the intestine. This is either "via antigen/sensitised mast cell combination or directly through secretion of a degranulation substance" which in turn "could recruit the eosinophils seen in the intestine after challenge". However, these authors were not able to resolve the question as to whether the role of the eosinophils in the intestine is only to "damp down" an anaphylactic reaction or whether they are involved in the destruction of immature flukes. Hughes, Harness and Doy (1977a) suggested both that resistance in rats is associated with immature stages and that mature flukes are either incapable of producing the right antigens or that their situation in the bile ducts prevents the hosts' response to such antigens. The latter suggestion is perhaps a more likely explanation of their observations since the present

investigation has shown that mature flukes implanted into rats are capable of stimulating significant resistance against oral challenge and that this is so even when the flukes are encapsulated in a permeable membrane.

Recently a new variant of the hypothesis which implicates immature flukes was suggested by Goose (1978). He found that the excretory and secretory products (ESP) of mature F. hepatica maintained in vitro were toxic to rat lymphocytes. There was a considerable decrease in the viability of these cells when they were incubated in the presence of ESP at 37°C for 2 hours. This phenomenon was employed in investigating the mechanism by which F. hepatica evades the host defences using an in vitro immunocytoadhesion system. When juvenile flukes were incubated with serum and peritoneal cells from rats resistant to F. hepatica, these flukes became heavily coated with cells which included many eosinophils. No cellular adhesion occurred when serum from un-infected rats was used. Inclusion of ESP in a system containing immune rat serum significantly reduced the number of flukes with adherent cells. Hence it was suggested that cellular attachment is the first step in the lethal immune reaction to F. hepatica and that, in vivo, ESP may prevent this attachment.

Continuing this line of thought, the author suggested that very young flukes would be expected to be the immunogenic stage of infection because they may not produce effective quantities of ESP while, in contrast, older, actively feeding flukes would be non-immunogenic. The author also suggested

that this hypothesis would explain the "early development of resistance after initial infection, the waning resistance in long-standing infections, the lack of resistance after implantation with adult flukes" and the importance of transferring serum in passive immunity experiments very soon after challenge before the production of effective quantities of ESP.

However, this hypothesis does not explain why a single primary infection which was allowed to mature appeared to result in a similar degree of resistance to oral challenge as two immature primary infections in rats in the present work (Table 4.2.).

The fact that metabolic products from mature flukes also appeared to be capable of stimulating resistance against challenge in the present work clearly conflicts with Goose's conclusion that mature flukes are non-immunogenic. However, although both immature and mature flukes can stimulate resistance, it is possible that different immunological mechanisms are stimulated by these.

Another consideration is the importance of a 'time factor' in resistance to F. hepatica, which was emphasised by Thorpe and Broome (1962). They reported that a period of 7 - 11 weeks after sensitisation with irradiated metacercariae was necessary for resistance to develop in rats. This was not associated with the maturity of flukes, since the irradiated metacercariae died before maturity. Their deduction was

supported by the observation by Corba, Armour, Roberts and Urquart (1971) that lymphoid cells from donor rats with a 4-week-old infection were not protective while those from donors with a 10-week-old infection were protective against challenge. Also Doyle (1973) found that whereas there was no significant resistance to challenge in calves with a 7-week-old primary infection, a significant resistance was obtained from a 12-week-old primary infection. He excluded the possibility that this resistance was due to the physical barrier of fibrosis or cholangitis since it is known that there is already fibrosis in the livers of calves infected with F. hepatica for 7 weeks (Dow, Ross and Todd, 1967). Thus the level of resistance increased with the increase in the duration of primary infection. Hence, Doyle also suggested that a 'time factor' is involved in the host's ability to develop a protective immune response. It was for this reason that the sera used in the passive transfer studies in the present work were usually obtained from donors which had been infected for 8-12 weeks. However one of the observations in the present study would seem to suggest that the time factor is not critical, at least in relation to the humoral protection arising from infection with adult flukes, as a significant level of resistance to challenge was obtained in rats which had been implanted with mature flukes encapsulated in diffusion chambers for 2 weeks.

As previously mentioned, the antigens from mature flukes might be less effective immunogens in natural infection because the flukes are in the bile ducts. In this respect

it would be interesting to see whether the bile in infected animals contains some of these antigens involved in resistance, especially as Gajos (1968) demonstrated specific antibody in bile against an antigen prepared from adult flukes, and Lehner (1977) showed that precipitating antigens in a Fasciola-metabolic-product and bile from infected rabbits show reactions of identity in immunodiffusion tests against infected rabbit serum.

The fact that serum gamma-globulin was found to be protective against F. hepatica also invites further research to determine the immunoglobulin class(es) involved in this resistance. Armour and Dargie (1973) have already reported that IgG but not IgM is involved in this resistance, whereas Lang (1974a) suggested that in mice both IgG and IgM are involved and that IgM possibly combines with a cell membrane antigen activating complement to produce immune lysis of fluke cells.

The specific antigens which stimulate the protective response also remain to be determined. Furthermore, the questions of why metabolic products collected from flukes maintained in vitro stimulated only a non-protective serologically detectable response (Lehner, 1977) while metabolic products released in vivo stimulated a protective response and the reason why such protection is not developed actively in rabbits and sheep also need to be elucidated.

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Appendex Table 2.1

The effect of varying the dilution of the enzyme-labelled anti-immunoglobulin in the assay of rat serum using a serum dilution of 1:25 and an antigen concentration of 4.5 mg/ml

Substrate	Anti-immunoglobulin dilution	Absorbance	
		Negative serum	Positive serum
OPD	1:50	0.08	0.78
	1:100	0.06	0.73
	1:200	0.05	0.62
	1:400	0.04	0.56
5AS	1:50	0.49	1.38
	1:100	0.48	0.67
	1:200	0.48	0.55
	1:400	0.43	0.48

Appendix Table 2.2

The effect of varying the dilution of the enzyme-labelled anti-immunoglobulin in the assay of rabbit serum using a serum dilution of 1:50 and an antigen concentration of 4.5 mg/ml

Substrate	Anti-immunoglobulin dilution	Absorbance	
		Negative serum	Positive serum
OPD	1:50	0.31	2.84
	1:100	0.28	2.66
	1:200	0.25	2.31
	1:400	0.23	1.82
5AS	1:50	0.41	1.80
	1:100	0.36	1.24
	1:200	0.32	0.86
	1:400	0.21	0.73

Appendix Table 2.3

The effect of varying the concentration of the antigen in the assay of rat serum using a conjugate dilution of 1:400, serum dilution of 1:25 and OPD in the substrate

Ag concentration (mg/ml)	Absorbance	
	Negative serum	Positive serum
00	00	00
0.12	0.05	0.15
3.2	0.05	0.36
4.5	0.12	0.78
7.9	0.20	0.82
15.8	0.24	1.42

Appendix Table 2.4

The effect of varying the concentration of the antigen in the assay of rabbit serum using a conjugate dilution of 1:400, serum dilution of 1:50 and OPD in the substrate

Ag concentration (mg/ml)	Absorbance	
	Negative serum	Positive serum
00	00	00
0.12	0.22	0.51
3.2	0.30	0.88
4.5	0.34	1.28
7.9	0.56	2.00

Appendix Table 2.5

The effect of varying the serum dilution in the assay of rat serum using a conjugate dilution of 1:400, Ag concentration of 4.5 mg/ml and OPD in the substrate

Serum dilution	Absorbance	
	Negative serum	Positive serum
1:25	0.06	0.74
1:50	0.04	0.66
1:100	0.02	0.48
1:200	00	0.41

Appendix Table 2.6

The effect of varying the serum dilution in the assay of rabbit serum using a conjugate dilution of 1:400, Ag concentration of 4.5 mg/ml and OPD in the substrate

Serum dilution	Absorbance	
	Negative serum	Positive serum
1:25	0.42	1.86
1:50	0.31	1.62
1:100	0.27	1.24
1:200	0.25	1.06
1:400	0.20	0.87

Appendix Table 4.1

Peripheral eosinophil counts (cells per mm^3) of rats treated with deacetylated diamphenethide to eliminate immature (Group A), mature (Group B) *F. hepatica* and infection controls (Group C)

Rat No.		0 w	2 w.p.i.	4 w.p.i.	6 w.p.i.	8 w.p.i.	10 w.p.i.
A	1	94	897	2417	597	922	119
	2	127	1395	1926	551	765	804
	3	109	78	2817	1306	412	413
	4	129	1803	2978	962	171	343
mean ±		115	1043	2535	854	568	420
s.d.		±16	±743	±469	±353	±340	±285
B	1	68	1701	2754	914	858	120
	2	226	1333	3018	3444	1057	160
	3	147	1029	1180	1251	306	201
	4	107	2316	2612	2890	1438	288
mean ±		137	1595	2391	2125	915	192
s.d.		±68	±554	±825	±1232	±472	±72
C	1	109	1101	2813	2245	1060	1089
	2	98	1734	4269	1529	1314	789
	3	85	1291	3921	2945	2444	790
	4	100	1202	2047	2030	1641	950
mean ±		98	1332	3263	2187	1615	905
s.d.		±10	±279	±1021	±588	±502	±144

Appendix Table 4.2

Serum glutamic dehydrogenase assays (micromoles product per litre of serum per minute) of rats treated with deacetylated diamphenethide to eliminate immature (Group A), mature (Group B) *F. hepatica* and infection controls (Group C)

Rat No.		0 w	2 w.p.i.	4 w.p.i.	6 w.p.i.	8 w.p.i.	10 w.p.i.
A	1	4.83	14.49	28.98	6.04	2.42	6.04
	2	5.33	9.66	24.15	8.70	4.24	4.83
	3	4.83	7.25	28.98	7.25	3.63	2.42
	4	4.83	8.70	21.79	4.83	4.83	4.83
mean ±		4.96	10.03	25.98	6.71	3.78	4.53
s.d.		±0.24	±3.14	±3.60	±1.66	±1.03	±1.52
B	1	5.33	12.08	28.98	14.49	9.66	7.25
	2	7.24	21.54	38.64	28.98	14.49	7.25
	3	3.62	19.32	38.64	38.64	19.32	6.04
	4	5.33	14.49	24.15	28.28	12.07	4.83
mean ±		5.38	16.86	32.60	27.60	13.89	6.34
s.d.		±1.48	±4.34	±7.24	±9.94	±4.13	±1.16
C	1	2.42	12.08	38.64	28.98	14.66	9.66
	2	9.66	19.32	28.98	24.15	12.07	4.83
	3	7.25	20.48	38.64	28.98	24.15	9.66
	4	7.25	7.18	38.64	28.98	8.45	9.66
mean ±		6.65	14.77	36.23	27.77	14.83	8.45
s.d.		±3.04	±6.28	±4.83	±2.41	±6.71	±2.42

Appendix Table 4.3

Peripheral eosinophil counts (cells per mm^3) of rats challenged after 2 previous infections and treatments Group a), challenged after treatment of a mature infection (Group B), challenge controls (Group C), first infection controls (Group D_1) and second infection controls (Group D_2)

Rat No.		0 w *	2 w	4 w	6 w *	8 w	10 w	12 w*	14 w	16 w	18 w	20 w
A	1	104*	716	1943	1475*	245	562	217*	1170	858	1693	1547
	2	118*	786	2110	274*	202	681	112*	464	526	1928	618
	3	00*	1047	2710	433*	725	1238	112*	420	401	722	848
	4	00*	2323	2536	256*	622	1147	254*	246	1252	832	734
	5	124*	484	2103	1635*	383	874	190*	564	1411	2611	2396
	6	102*	788	4754	306*	239	683	214*	293	100	1415	1070
	7	00*	1557	3447	304*	715	842	559*	1123	2360	3012	1215
	8	395*	2039	1873	1130*	2666	1594	296*	446	617	1603	1307
	9	255*	1887	2436	1228*	2610	1446	127*	126	763	1617	1428
	10	171*	929	3646	753*	992	933	325*	1242	1072	990	840
	11	354*	1156	2074	379*	1123	1716	128*	365	1532	2332	1354
	12	138*	1040	1494	262*	248	754	535*	860	1328	2605	951
	13	149*	3388	3030	2516*	350	825	248*	1349	2640	3705	1610
	14	152*	402	399	247*	699	1001	124*	694	587	1111	1492
mean ±		147*	1324	2468	799*	844	1021	246*	668	1103	1870	1243
s.d.		±119	±831	±1046	±699	±813	±358	±145	±407	±723	±881	±460
B	1	228*	462	268	924	665	496	408*	1610	824	840	954
	2	226*	3366	2548	4592	765	380	460*	249	800	388	1118
	3	633*	4180	3960	3916	2220	2142	1091*	1283	464	1508	2033
	4	130*	1368	1664	1210	595	648	819*	999	920	1175	2261
	5	232*	2226	3150	2472	1738	1467	980*	1080	1785	1235	1746
	6	00*	1824	2754	1944	600	444	288*	340	1089	987	808
	7	184*	1308	2860	2261	931	1404	864*	1552	776	712	864
	8	00*	2125	2408	1112	1529	1664	00 *	380	1014	946	1248
	9	99*	2736	3042	2958	840	204	91*	540	288	760	690
	10	113*	246	450	555	568	198	97*	742	2280	4380	1845
	11	264*	1800	2864	2002	1008	77	255*	680	1026	1584	861
	12	135*	2880	4212	3979	1815	1526	610*	2235	3888	5616	2235
mean ±		186*	2043	2515	2327	1106	887	496*	974	1262	1677	1388
s.d.		±164	±1143	±1205	±1310	±568	±704	±371	±606	±986	±1608	±594
C	1	387	264	250	324	198	460	242*	1088	1464	2205	1443
	2	248	238	472	452	868	324	339*	1988	2130	2465	2850
	3	278	420	264	426	588	274	387*	1386	2160	2844	2037
	4	495	234	212	291	336	220	345*	1332	1443	1017	739
	5	125	242	234	285	188	201	297*	900	2086	2669	2599
	6	268	396	216	198	356	440	363*	2898	2304	4212	5454
	7	360	252	366	488	436	254	270*	2535	3792	4806	1496
	8	196	198	202	111	00	214	357*	3475	2256	1840	1488
mean ±		294	280	277	321	371	298	325*	1950	2204	2757	2263
s.d.		±116	±81	±94	±129	±267	±101	±50	±934	±725	±1230	±1457
D_1	1	135*	696	1584	1848	296						
	2	465*	548	1326	1395	802						
	3	00*	525	1332	2431	432						
	4	162*	966	2562	1395	1140						
mean ±		190*	683	1701	1767	667						
s.d.		±196	±202	±586	±491	±380						
D_2	1	89*	1529	1953	1980	300						
	2	96*	1632	1589	2058	304						
	3	110*	1368	1323	2856	455						
	4	196*	1683	2445	1200	106						
mean ±		122*	1553	1827	2023	291						
s.d.		±49*	±139	±486	±676	±143						

* Administration of infection

Appendix Table 4.4

Serum glutamic dehydrogenase assays (micromoles product per litre of serum per minute) of rats challenged after 2 previous infections and treatments (Group A), challenged after treatment of a mature infection (Group B), challenge controls (Group C), first infection controls (Group D_1) and second infection controls (Group D_2)

Rat No.		0 w*	2 w	4 w	6 w*	8 w	10 w	12 w*	14 w	16 w	18 w	20 w
A	1	2.42*	9.66	48.30	14.49*	14.49	16.91	10.87*	14.49	16.91	16.91	9.66
	2	4.83*	14.49	38.70	9.66*	14.49	14.49	7.25*	7.25	9.66	9.66	9.66
	3	4.83*	9.66	24.15	14.49*	12.08	14.49	7.25*	7.25	7.25	4.83	4.83
	4	2.42*	9.66	33.81	16.91*	19.32	24.15	14.49*	14.49	24.15	24.15	9.66
	5	2.42*	14.49	48.30	18.98*	19.32	19.32	14.49*	14.49	19.32	16.91	14.49
	6	4.83*	14.49	30.19	14.49*	16.49	24.15	12.08*	14.49	14.49	14.49	9.66
	7	3.14*	11.59	57.96	24.15*	24.15	19.32	16.91*	16.91	19.49	19.49	9.66
	8	3.38*	9.66	33.81	9.66*	14.49	19.32	7.24*	9.66	9.66	12.08	9.66
	9	4.83*	16.91	48.30	14.49*	14.49	38.81	7.24*	12.08	16.91	24.16	14.49
	10	4.83*	9.66	48.30	14.49*	14.49	24.15	9.66*	14.49	16.91	14.49	4.83
	11	3.38*	9.66	28.98	8.46*	16.91	16.91	9.66*	10.87	12.08	16.91	9.66
	12	4.83*	14.49	33.81	14.49*	16.91	33.81	12.08*	14.49	17.87	12.08	9.66
	13	4.83*	9.66	48.30	13.52*	9.66	9.66	9.66*	12.08	24.16	24.15	16.91
	14	2.42*	14.49	48.30	16.91*	24.15	33.81	14.49*	14.49	14.49	9.66	4.83
mean ±		3.81*	12.04	48.80	14.66*	16.75	21.74	10.96*	12.68	15.95	15.71	9.83
s.d.		±1.10	±2.68	±10.05	±4.01	±4.19	±7.69	±3.23	±2.95	±5.11	±5.91	±3.60
B	1	2.42*	9.66	24.15	33.81	14.49	9.66	7.25*	12.07	16.90	14.49	9.66
	2	4.83*	12.07	53.15	44.26	38.64	21.74	9.66*	14.49	24.15	24.15	14.49
	3	4.83*	9.66	66.91	62.79	24.15	9.66	9.66*	12.08	14.49	21.74	9.66
	4	2.42*	7.24	24.15	24.15	9.66	9.66	7.25*	12.08	14.49	16.91	9.66
	5	4.83*	9.66	53.13	50.71	26.56	12.08	12.08*	14.49	24.15	16.91	9.66
	6	2.42*	12.07	33.81	26.56	7.25	7.25	4.83*	7.25	12.08	14.49	9.66
	7	4.83*	9.66	24.15	15.70	7.25	7.25	4.83*	7.25	9.66	14.49	4.83
	8	2.42*	18.11	53.13	50.71	21.74	14.49	9.66*	12.08	21.74	24.15	14.49
	9	4.83*	16.90	43.47	26.56	7.25	4.83	4.83*	9.66	14.49	14.49	9.66
	10	4.83*	4.83	14.49	16.91	12.07	3.62	4.83*	14.49	14.49	16.91	9.66
	11	3.62*	14.49	43.47	43.47	14.49	14.49	4.83*	9.66	14.49	16.91	14.49
	12	4.83*	7.24	33.81	30.19	24.15	9.66	9.66*	14.49	16.91	24.15	16.91
mean ±		3.93*	10.97	38.98	35.49	17.31	10.37	7.45*	11.67	16.50	18.32	11.07
s.d.		±1.16	±3.97	±15.77	±14.79	±9.81	±4.90	±2.62	±2.69	±4.59	±4.05	±3.33
C	1	4.83	4.83	4.83	4.83	4.83	4.83	4.83*	1.66	24.15	33.81	9.66
	2	2.42	3.61	3.61	4.83	3.61	3.61	4.83*	4.83	19.32	26.57	19.32
	3	2.42	3.61	3.61	4.83	3.61	3.61	3.61*	14.49	33.81	33.81	14.49
	4	3.61	3.61	3.61	4.83	4.83	4.83	4.83*	9.66	19.32	24.15	14.49
	5	4.83	4.83	4.83	4.83	4.83	4.83	4.83*	9.66	26.57	33.81	9.66
	6	2.42	4.83	2.42	2.42	2.42	2.42	2.42*	14.49	26.57	33.81	9.66
	7	4.83	3.61	3.61	3.61	4.83	3.61	3.61*	14.49	48.30	26.57	14.49
	8	4.83	4.83	3.61	3.61	4.83	4.83	4.83*	12.08	33.81	24.15	14.49
mean ±		3.77	4.22	3.77	4.22	4.22	4.07	4.22*	11.17	28.98	29.59	13.28
s.d.		±1.20	±0.65	±0.77	±0.92	±0.92	±0.90	±0.92	±3.14	±9.57	±4.61	±3.42
D_1	1	4.83*	12.08	24.15	28.98	9.66						
	2	2.42*	9.66	28.98	33.81	14.49						
	3	2.42*	9.66	33.81	48.30	19.32						
	4	4.83*	9.66	19.32	24.15	9.66						
mean ±		3.63*	10.27	26.57	33.81	13.28						
s.d.		±1.39	±1.21	±6.24	±10.43	±4.62						
D_2	1	4.83*	14.49	33.81	33.81	14.49						
	2	2.42*	7.25	28.98	24.15	9.66						
	3	2.42*	9.66	24.15	26.56	9.66						
	4	2.42*	14.49	33.81	48.30	14.49						
mean ±		3.02*	11.47	30.19	33.21	12.08						
s.d.		±1.21	±3.62	±4.62	±10.87	±2.79						

*Administration of infection

Appendix Table 4.5

ELISA values (units of absorbance) of rats challenged after two previous infections, and treatments (Group A), challenged after treatment of a mature infection (Group B) and challenge controls (Group C)

Rat No.		0 w*	2 w	4 w	6 w *	8 w	10 w	12 w*	14 w	16 w	18 w	20 w
A	1	0.12*	0.20	0.24	0.40*	0.52	0.56	0.78*	0.80	0.80	0.86	0.74
	2	0.18*	0.24	0.21	0.46*	0.58	0.62	0.68*	0.84	0.74	0.75	0.72
	3	0.15*	0.23	0.33	0.54*	0.58	0.80	0.72*	0.72	0.72	0.65	0.64
	4	0.22*	0.28	0.34	0.40*	0.48	0.57	0.61*	0.63	0.68	0.74	0.90
	5	0.20*	0.36	0.37	0.48*	0.58	0.43	0.56*	0.64	0.70	0.70	0.68
	6	0.15*	0.23	0.29	0.53*	0.66	0.62	0.68*	0.70	0.66	0.59	0.61
	7	0.20*	0.31	0.33	0.35*	0.42	0.52	0.64*	0.68	0.69	0.68	0.55
	8	0.22*	0.40	0.42	0.49*	0.53	0.56	0.62*	0.72	0.74	0.76	0.72
	9	0.12*	0.41	0.47	0.56*	0.60	0.53	0.64*	0.70	0.71	0.72	0.76
	10	0.08*	0.12	0.34	0.41*	0.54	0.56	0.68*	0.72	0.74	0.80	0.78
	11	0.09*	0.18	0.30	0.48*	0.55	0.58	0.82*	0.90	0.90	0.92	0.78
	12	0.10*	0.29	0.40	0.46*	0.56	0.56	0.76*	0.64	0.70	0.72	0.68
	13	0.16*	0.23	0.30	0.41*	0.60	0.62	0.62*	0.52	0.60	0.90	0.80
	14	0.04*	0.20	0.24	0.56*	0.54	0.58	0.64*	0.66	0.74	0.76	0.72
mean ±		0.07*	0.26	0.33	0.47*	0.55	0.58	0.68*	0.71	0.72	0.75	0.72
s.d.		±0.08	±0.08	±0.07	±0.07	±0.06	±0.08	±0.07	±0.09	±0.07	±0.09	±0.09
B	1	0.08*	0.11	0.34	0.52	0.58	0.58	0.60*	0.68	0.72	0.78	0.74
	2	0.06*	0.19	0.36	0.39	0.56	0.54	0.62*	0.60	0.64	0.70	0.70
	3	0.10*	0.23	0.30	0.46	0.55	0.48	0.60*	0.70	0.72	0.76	0.74
	4	0.09*	0.19	0.32	0.42	0.52	0.62	0.58*	0.72	0.68	0.72	0.64
	5	0.10*	0.20	0.28	0.42	0.48	0.54	0.68*	0.74	0.78	0.80	0.74
	6	0.08*	0.17	0.24	0.38	0.48	0.42	0.42*	0.60	0.72	0.74	0.68
	7	0.15*	0.23	0.34	0.44	0.52	0.48	0.56*	0.68	0.74	0.68	0.63
	8	0.12*	0.21	0.32	0.43	0.62	0.54	0.58*	0.72	0.70	0.78	0.78
	9	0.09*	0.18	0.30	0.40	0.56	0.52	0.62*	0.74	0.68	0.72	0.78
	10	0.09*	0.20	0.26	0.45	0.51	0.52	0.64*	0.76	0.78	0.74	0.70
	11	0.10*	0.23	0.39	0.44	0.54	0.48	0.56*	0.60	0.74	0.70	0.64
	12	0.11*	0.24	0.38	0.43	0.54	0.46	0.58*	0.69	0.70	0.72	0.70
mean ±		0.10*	0.20	0.32	0.43	0.54	0.52	0.59*	0.69	0.72	0.74	0.71
s.d.		±0.02	±0.04	±0.05	±0.04	±0.04	±0.05	±0.06	±0.06	±0.04	±0.04	±0.05
C	1	0.12*	0.41	0.47	0.42	0.60						
	2	0.19*	0.20	0.44	0.54	0.50						
	3	0.09*	0.21	0.24	0.42	0.48						
	4	0.16*	0.33	0.45	0.60	0.52						
	5	0.10*	0.21	0.34	0.48	0.56						
	6	0.14*	0.32	0.34	0.52	0.54						
	7	0.08*	0.16	0.29	0.38	0.48						
	8	0.08*	0.14	0.38	0.53	0.44						
mean ±		0.12*	0.25	0.37	0.49	0.52						
s.d.		±0.04	±0.09	±0.08	±0.07	±0.05						

* Administration of infection

Appendix Table 4.6

Peripheral eosinophil counts (cells per mm^3) of rabbits with 2 previous infections and treatments (Group A), challenge controls (Group B), first infection controls (Group C) and second infection controls (Group D)

Rabbit No.		0 w*	2 w	4w	6 w *	8 w	10 w	12 w*	14 w	16 w	18 w	20 w
A	1	170*	875	2234	563*	3307	1316	794*	405	1716	2194	2149
	2	204*	2667	4416	6271*	3893	11046	748*	2143	3387	4909	4904
	3	81*	685	1709	922*	4832	3262	1126*	1619	1944	2595	1087
	4	256*	1418	2221	1815*	4041	5423	2030*	810	1725	2308	1959
	5	78*	1016	3028	667*	4345	2787	1484*	824	1779	1979	2985
	6	245*	1461	1534	1096*	5701	4341	1412*	1297	2163	2550	6764
mean ±		172*	1354	2524	1889*	4353	4696	1266*	1183	2119	2756	3308
s.d.		±78	±711	±1063	±2192	±831	±3409	±482	±632	±644	±1079	±2130
B	1	00	188	00	00	292	155	290*	141	1708	2204	3787
	2	145	00	00	119	146	248	250*	433	843	2175	2411
	3	85	218	00	105	00	00	296*	326	3294	5946	5304
	4	268	344	151	190	352	148	324*	473	3002	1742	3091
	5	254	157	318	446	308	260	270*	00	934	2081	2421
	6	210	324	330	180	578	531	338*	364	4500	3737	2192
	7	105	353	00	00	107	290	214*	486	166	1773	1819
mean ±		152	226	114	149	255	233	283*	318	2291	2808	3004
s.d.		±98	±127	±154	±152	±190	±164	±43	±183	±1371	±1539	±1202
C	1	194*	527	1843	3763	2772						
	2	110*	697	2673	5840	3772						
mean ±		152*	612	2258	4802	3272						
s.d.		±59	±120	±587	±1469	±707						
D	1	00*	430	2388	1966	1274						
	2	148*	89	1636	2242	1321						
mean ±		74*	260	2012	2104	1297						
s.d.		±105	±241	±532	±195	±33						

* Administration of infection

Appendix Table 4.7

Serum glutamic dehydrogenase assays (micromoles product per litre of serum per minute) of rabbits with 2 previous infections and treatments (Group A), challenge controls (Group B), first infection controls (Group C) and second infection controls (Group D)

Rabbit No.		0 w*	2 w	4 w	6 w *	8 w	10 w	12 w *	14 w	16 w	18 w	20 w
A	1	3.62*	9.66	19.32	24.15*	36.23	28.98	12.08*	8.45	12.08	–	9.66
	2	4.83*	12.08	19.32	16.91*	14.49	16.91	7.25*	12.08	12.08	8.45	7.25
	3	7.25*	15.70	15.70	24.15*	14.49	14.49	4.83*	9.66	12.08	14.49	9.66
	4	8.45*	19.32	21.44	9.66*	24.15	24.15	14.49*	9.66	12.08	14.49	7.25
	5	4.83*	7.25	16.91	14.49*	14.49	28.98	4.83*	–	–	7.25	4.83
	6	2.42*	14.49	16.91	16.91*	28.98	28.98	14.49*	19.32	–	19.32	16.91
mean ±		5.23*	13.08	18.27	17.71*	22.14	23.75	9.66*	11.83	12.08	12.80	9.26
s.d.		±2.25	±4.35	±2.12	±5.65	±9.22	±6.55	±4.58	±4.39	±00	±4.95	±4.16
B	1	2.42	2.42	2.42	2.42	2.42	2.42	3.63*	9.66	19.32	25.36	14.49
	2	2.42	2.42	2.42	2.42	2.42	2.42	2.42*	4.83	25.36	25.36	19.32
	3	2.42	2.42	2.42	2.42	2.42	2.42	2.42*	6.04	25.36	25.36	9.66
	4	2.42	2.42	2.42	2.42	1.21	2.42	3.63*	9.66	16.91	24.15	7.25
	5	1.21	1.21	1.21	2.42	1.21	2.42	4.83*	4.83	16.91	24.15	21.74
	6	1.21	1.21	1.21	2.42	1.21	2.42	4.83*	9.66	16.91	19.32	19.32
	7	1.21	2.42	1.21	2.42	1.21	2.42	3.63*	9.66	25.36	25.36	7.25
mean ±		1.90	2.07	1.90	2.42	1.73	2.42	3.63*	7.76	20.88	24.15	14.15
s.d.		±0.42	±0.59	±0.65	±00	±0.65	±00	±0.98	±2.40	±4.28	±2.20	±6.14
C	1	00 *	4.83	19.32	19.32	12.08						
	2	2.42*	6.04	19.32	25.36	21.72						
mean ±		1.21*	5.44	19.32	22.34	16.90						
s.d.		±1.71	±0.85	±00	±4.27	±6.82						
D	1	3.63*	6.04	25.36	18.12	14.49						
	2	2.42*	3.63	13.29	32.61	19.32						
mean ±		3.03*	4.84	19.33	25.37	16.91						
s.d.		±0.86	±1.70	±8.53	±10.25	±3.41						

* Administration of infection

Appendix Table 4.8

ELISA values (units of absorbance) of rabbits with 2 previous infections and treatments (Group A), challenge controls (Group B), first infection controls (Group C) and second infection controls (Group D)

Rabbit No.		0 w*	2 w	4 w	6 w*	8 w	10 w	12 w*	14 w	16 w	18 w	20 w
A	1	0.39*	0.74	1.48	1.60*	2.68	2.78	2.82*	2.70	2.82	2.80	2.80
	2	0.34*	0.75	1.36	2.04*	2.40	2.52	2.96*	2.60	2.74	2.60	2.60
	3	0.33*	1.01	1.18	1.64*	2.30	2.50	2.80*	3.60	2.98	2.68	2.88
	4	0.44*	0.90	1.01	1.70*	2.10	2.36	2.84*	2.76	2.82	2.85	3.60
	5	0.50*	0.98	1.48	1.76*	2.84	3.20	3.60*	2.88	2.86	2.73	3.60
	6	0.35*	0.96	1.68	2.52*	2.84	2.90	2.92*	2.60	2.66	3.60	3.60
mean ±		0.39*	0.89	1.37	1.88*	2.53	2.71	2.99*	2.69	2.81	2.88	3.18
s.d.		±0.07	±0.12	±0.24	±0.35	±0.31	±0.31	±0.31	±0.11	±0.11	±0.36	±0.47
B	1	0.15	0.20	0.10	0.15	0.20	0.20	0.25*	0.60	2.25	1.75	2.20
	2	0.15	0.15	0.20	0.15	0.25	0.20	0.20*	0.60	1.40	1.85	1.25
	3	0.50	0.50	0.40	0.45	0.35	0.35	0.35*	1.05	1.15	1.20	1.25
	4	0.50	0.30	0.35	0.35	0.35	0.35	0.40*	0.65	0.90	1.50	2.30
	5	0.10	0.20	0.25	0.25	0.20	0.25	0.25*	0.65	0.95	1.15	1.80
	6	0.16	0.15	0.20	0.25	0.25	0.20	0.25*	0.60	0.90	1.55	1.95
	7	0.15	0.15	0.21	0.15	0.20	0.20	0.15*	0.65	0.95	1.35	1.50
mean ±		0.24	0.24	0.24	0.25	0.26	0.25	0.26*	0.69	1.21	1.48	1.75
s.d.		±0.17	±0.14	±0.10	±0.11	±0.07	±0.07	±0.09	±0.17	±0.49	±0.26	±0.42
C	1	0.42*	0.88	2.24	1.78	1.88						
	2	0.44*	0.98	1.84	2.18	2.06						
mean ±		0.43*	0.93	2.04	1.98	1.97						
s.d.		±0.01	±0.07	±0.28	±0.28	±0.13						
D	1	0.43*	0.96	1.01	1.36	1.72						
	2	0.30*	0.86	1.28	1.80	1.88						
mean ±		0.37*	0.91	1.15	1.58	1.80						
s.d.		±0.09	±0.07	±0.19	±0.31	±0.11						

* Administration of infection

Appendix Table 4.9

Peripheral eosinophil counts (cell per mm^3) of rabbits which were challenged after treatment of a mature infection (Group A) and challenge controls (Group B)

Rabbit No.		0 w	2 w.p.i.	4 w.p.i.	6 w.p.i.	8 w.p.i.
A	1	00	974	535	2670	2040
	2	625	1536	2331	2128	2772
	3	404	1330	1365	744	1734
	4	00	546	1212	2016	1717
	5	216	360	786	2299	1717
	6	00	976	1375	2928	2052
mean ± s.d.		207±261	953±447	1267±620	2130±760	2005±407
B	1	116	525	819	894	1113
	2	00	736	336	795	1155
	3	476	960	2448	2224	868
	4	00	520	714	3640	1833
	5	00	1287	1456	3480	2355
	6	00	873	1019	2904	1768
	7	00	510	468	1912	1326
	8	00	438	720	424	2256
mean ±s.d.		74±167	731±293	997±678	2034±1248	1584±551

Appendix Table 4.10

Serum glutamic dehydrogenase assays (micromoles product per litre of serum per minute) of rabbits which were challenged after treatment of a mature infection (Group A) and challenge controls (Group B)

Rabbit No.		0 w	2 w.p.i.	4 w.p.i.	6 w.p.i.	8 w.p.i.
A	1	4.83	9.66	24.15	24.15	9.66
	2	4.83	14.49	28.98	48.30	9.66
	3	2.42	14.49	14.49	24.15	19.32
	4	2.42	9.66	33.81	48.30	9.66
	5	00	4.83	24.15	28.98	7.25
	6	4.83	9.66	33.81	24.15	00
mean ± s.d.		3.22±1.97	10.47±3.64	26.57±7.33	33.01±11.99	9.26±6.19
B	1	2.42	9.66	19.32	33.81	19.32
	2	2.42	9.66	19.32	33.81	9.66
	3	2.42	7.25	28.98	33.81	16.91
	4	00	4.83	19.32	24.15	2.42
	5	2.42	7.25	19.32	48.30	9.66
	6	2.42	14.49	19.49	19.32	19.32
	7	4.83	9.66	14.49	24.15	7.25
	8	4.83	14.49	30.64	63.29	19.32
mean ± s.d.		2.72±1.55	9.66±3.41	20.74±6.00	35.08±14.46	12.98±6.57

Appendix Table 5.1

Peripheral eosinophil counts (cells per mm^3) of rats implanted s/c with living flukes before challenge (Group A) and infection controls (Group B)

Rat No.		0 w	2 w.a.i.	4 w.a.i.	6 w.a.i.	8 w.a.i.
A	1	1040	178	302	00	816
	2	1298	380	1368	129	284
	3	490	355	2736	1072	475
	4	152	292	570	122	678
	5	1647	477	1062	314	920
	6	576	783	96	00	1364
	7	1120	1725	324	121	520
	8	430	336	1380	1536	1932
	9	96	1164	1834	1859	1740
	10	94	380	1224	396	776
	11	91	1397	1727	2405	762
	12	99	246	1261	2414	1802
mean ± s.d.		594±548	642±510	1157±756	864±949	1005±561
B*	1	00	816	1525	1419	1704
	2	186	981	229	2145	685
	3	90	208	600	678	172
	4	231	608	2832	3270	2814
	5	198	848	1464	1392	642
	6	68	737	2717	1134	972
	7	00	1062	1169	3195	1200
	8	79	405	1680	630	2044
mean ± s.d.		106±89	708±288	1527±911	1732±1040	1279±862

*Also used as an infection control group in experiment (6.1) carried out at the same time.

Appendix Table 5.2

Serum glutamic dehydrogenase assays (micromoles per litre of serum per minute) of rats implanted s/c with living flukes before challenge (Group A) and infection controls (Group B)

Rat No.		0 w	2 w.a.i.	4 w.a.i.	6 w.a.i.	8 w.a.i.
A	1	2.42	2.42	00	2.42	00
	2	4.83	00	4.83	4.83	4.83
	3	4.83	2.42	28.98	9.66	9.66
	4	2.42	2.42	4.83	9.66	2.42
	5	2.42	4.83	4.83	4.83	4.83
	6	4.83	4.83	4.83	4.83	4.83
	7	4.83	4.83	7.25	2.42	2.42
	8	2.42	2.42	2.42	7.25	2.42
	9	4.83	4.83	24.15	24.15	28.98
	10	2.42	2.42	2.42	2.42	2.42
	11	4.83	2.42	28.98	19.32	9.66
	12	00	2.42	9.66	9.66	00
mean ± s.d.		3.42±1.61	3.02±1.50	10.27±10.66	8.45±6.87	6.04±7.88
B*	1	4.83	7.25	19.32	19.32	9.66
	2	00	7.25	19.32	19.32	9.66
	3	2.42	9.66	14.49	19.32	4.83
	4	2.42	9.66	43.47	14.49	9.66
	5	2.42	9.66	19.32	9.66	9.66
	6	4.83	9.66	43.47	48.30	9.66
	7	4.83	14.49	19.32	28.98	9.66
	8	2.42	7.25	14.49	14.49	4.83
mean ± s.d.		3.02±1.71	9.36±2.39	24.15±12.11	21.74±12.11	8.45±2.24

* Also used as an infection control group in experiment (6.1) carried out at the same time.

Appendix Table 5.3

Peripheral eosinophil counts (cells per mm^3) of rabbits implanted s/c with living flukes before challenge (Group A) and infection controls (Group B)

Rabbit No.		0 w	2 w.a.i.	4 w.a.i.	6 w.a.i.	8 w.a.i.
A	1	428	1785	570	1428	714
	2	424	945	3591	3318	1677
	3	792	1074	274	3759	1794
	4	987	1148	3381	2366	732
	5	605	966	2310	2112	1476
	6	408	980	2535	1824	1890
	7	292	808	2028	2197	1548
	8	715	1690	1885	2282	1375
mean ± s.d.		581±235	1174±362	2071±1184	2410±765	1400±450
B	1	497	869	2288	5096	2352
	2	260	776	1563	3388	2360
	3	575	1182	1409	3232	1932
	4	100	2166	2506	2669	2340
	5	1072	882	1200	2768	1710
mean ± s.d.		500±370	1175±574	1793±571	3430±979	2138±300

Appendix 5.4

Serum glutamic dehydrogenase (micromoles product per litre of serum per minute) of rabbits implanted s/c with living flukes before challenge (Group A) and infection controls (Group B)

Rabbit No.		0 w	2 w.a.i.	4 w.a.i.	6 w.a.i.	8 w.a.i.
A	1	4.83	19.32	19.32	33.81	9.66
	2	4.83	14.49	19.32	24.15	14.49
	3	00	24.15	24.15	19.32	9.66
	4	2.42	14.49	19.32	28.98	14.49
	5	2.42	19.32	24.15	19.32	9.66
	6	00	9.66	14.49	19.32	9.66
	7	2.42	9.66	14.49	19.32	9.66
	8	2.42	9.66	19.32	14.49	9.66
mean ± s.d.		1.81±1.71	15.09±5.44	19.32±3.65	22.34±6.29	10.87±2.24
B	1	4.83	24.15	24.15	14.49	9.66
	2	00	9.66	19.32	33.81	14.49
	3	00	9.66	14.49	19.32	9.66
	4	00	9.66	14.49	19.32	9.66
	5	00	9.66	14.49	14.49	9.66
mean ± s.d.		0.97±2.16	12.56±6.48	17.39±4.32	20.29±7.94	10.63±2.16

Appendix Table 5.5.

Serum glutamic dehydrogenase assays (micromoles product per litre of serum per minute) of rats implanted i/p with 1 or 2 mature flukes in chambers (Group A), rats implanted with empty chambers (Group B), non-implanted infection controls (Group C), rats implanted i/p with 1 or 2 dead flukes in chambers (Group D) and rats implanted i/p with 1 or 2 in mature flukes (Group E)

Rat No.		0 w.	2 w.a.i.	4 w.a.i.	6 w.a.i.	8 w.a.i.
A	1	4.83	4.83	9.66	14.49	4.83
	2	4.83	9.66	24.15	24.15	9.66
	3	4.83	9.66	14.49	19.32	14.49
	4	00	4.83	14.49	14.49	9.66
	5	4.83	19.32	24.15	19.32	9.66
	6	4.83	9.66	14.49	14.49	4.83
	7	4.83	14.49	28.98	14.49	00
	8	4.83	9.66	9.66	9.66	4.83
	9	9.66	14.49	25.81	19.32	9.66
	10	2.42	9.66	38.64	19.32	2.42
	11	4.83	9.66	14.49	14.49	4.83
	12	4.83	9.66	38.64	38.64	9.66
mean ± s.d.		4.63±2.17	10.47±4.03	21.47±10.24	18.52±7.38	7.04±4.05
B	1	4.83	14.49	38.64	48.30	28.98
	2	4.83	9.66	38.64	19.32	9.66
	3	4.83	9.66	19.32	19.32	9.66
	4	4.83	14.49	43.47	38.64	19.32
	5	4.83	9.66	43.47	14.49	19.32
	6	4.83	19.32	38.64	38.64	9.66
	7	4.83	19.32	38.64	38.64	9.66
	8	4.83	9.66	28.98	28.98	19.32
mean ± s.d.		4.83±00	13.28±4.28	36.23±8.16	30.79±12.09	15.70±7.19
C	1	4.83	19.32	48.30	48.30	19.32
	2	4.83	9.66	33.81	33.81	14.49
	3	4.83	19.32	53.13	19.32	19.32
	4	4.83	9.66	19.32	28.98	14.49
	5	4.83	14.49	33.81	14.49	9.66
	6	4.83	19.32	57.84	28.98	14.49
	7	00	4.83	33.81	33.81	9.66
mean ± s.d.		4.14±1.82	13.80±5.87	40.00±13.55	29.67±10.95	14.49±3.94
D	1	4.83	19.32	48.30	48.30	19.32
	2	4.83	19.32	38.64	28.98	9.66
	3	9.66	14.49	48.30	48.30	19.32
	4	00	9.66	48.30	38.64	9.66
	5	4.83	9.66	48.30	48.30	19.32
	6	4.83	14.49	57.84	48.30	19.32
mean ± s.d.		4.83±3.05	14.49±4.32	48.28±6.07	43.47±8.08	16.10±4.99
E	1	4.83	4.83	28.98	19.32	9.66
	2	4.83	9.66	33.81	19.32	9.66
	3	4.83	9.66	19.32	9.66	9.66
	4	4.83	4.83	19.32	4.83	4.83
	5	4.83	9.66	19.32	19.32	9.66
	6	4.83	19.32	19.32	9.66	4.83
mean ± s.d.		4.83±00	9.66±5.29	23.35±6.42	13.69±6.42	8.05±2.49

Appendix Table 5.6

Serum glutamic dehydrogenase assays (micromoles product per litre of serum per minute) of rats implanted s/c with 2 mature flukes in chambers which were removed before challenge (Group A), rats implanted s/c with 2 empty chambers which were removed before challenge (Group B), rats implanted with 2 mature flukes in chambers (Group C), rats implanted s/c with 2 empty chambers (Group D), rats implanted i/p with 2 mature flukes in chambers (Group E) and rats implanted i/p with empty chambers (Group F)

Rat No		0 w	2 w.a..i.	4 w.a.i.	6 w.a.i.	8 w.a.i.
A	1	4.83	4.83	4.83	4.83	4.83
	2	4.83	9.66	9.66	14.49	4.83
	3	4.83	4.83	4.83	14.49	9.66
	4	2.42	4.83	9.66	9.66	9.66
	5	4.83	4.83	9.66	9.66	9.66
	6	9.66	9.66	9.66	9.66	4.83
	7	4.83	14.49	14.49	28.98	9.66
	8	4.83	4.83	4.83	4.83	4.83
mean ± s.d.		5.13±2.01	7.25±3.65	8.45±3.42	12.08±7.75	7.25±2.58
B	1	9.66	14.49	33.81	38.64	9.66
	2	4.83	14.49	38.64	48.30	9.66
	3	4.83	9.66	38.64	38.64	14.49
	4	4.83	9.66	48.30	48.30	14.49
	5	4.83	14.49	24.15	19.32	9.66
	6	4.83	9.66	24.15	24.15	9.66
	7	9.66	9.66	48.30	38.64	9.66
	8	4.83	9.66	24.15	33.81	9.66
	9	4.83	14.49	38.64	33.81	14.49
mean ± s.d.		5.90±2.13	11.81±2.55	35.42±9.66	35.96±9.69	11.27±2.42
C	1	4.83	9.66	14.49	24.15	9.66
	2	4.83	9.66	28.98	28.98	9.66
	3	4.83	9.66	14.49	28.98	9.66
	4	4.83	14.49	14.49	14.49	9.66
	5	4.83	4.83	4.83	9.66	00
mean ± s.d.		4.83±00	9.66±3.42	15.46±8.64	21.25±8.77	7.73±4.32
D	1	9.66	9.66	24.15	24.15	4.83
	2	9.66	9.66	19.32	33.81	4.83
	3	4.83	9.66	24.15	24.15	9.66
	4	4.83	9.66	33.81	48.30	9.66
	5	4.83	9.66	48.30	48.30	9.66
mean ± s.d.		6.76±2.65	9.66±00	29.95±10.31	35.74±12.12	7.73±2.65
E	1	4.83	4.83	9.66	14.49	9.66
	2	4.83	9.66	14.49	14.49	9.66
	3	4.83	4.83	4.83	9.66	4.83
	4	4.83	9.66	14.49	14.49	9.66
	5	4.83	9.66	24.15	24.15	14.49
	6	2.42	2.42	4.83	2.42	4.83
	7	4.83	9.66	14.49	14.49	4.83
	8	4.83	4.83	4.83	9.66	9.66
mean ± s.d.		4.53±0.85	6.94±3.01	11.47±6.80	12.98±6.18	8.45±3.42
F	1	9.66	9.66	33.81	33.81	4.83
	2	4.83	9.66	33.81	33.81	9.66
	3	9.66	14.49	48.30	38.64	14.49
	4	4.83	9.66	19.32	19.32	4.83
	5	4.83	9.66	33.81	24.15	4.83
	6	4.83	9.66	38.81	38.64	9.66
	7	4.83	9.66	38.81	33.81	9.66
mean ± s.d.		6.21±2.36	10.35±1.83	35.24±8.71	31.74±7.3	8.28±3.65

Appendix Table 5.7

Serum glutamtic dehydrogenase assays (micromoles product per litre of serum per minute) of rats implanted i/p with 2 mature flukes in chambers twice at an interval of 12 weeks before challenge (Group A) and controls similarly implanted with empty chambers (Group B)

Rat No.		0 w.	2 w.a.i.	4 w a.i.	6 w.a.i.	8 w.a.i.
A	1	4.83	4.83	4.83	9.66	4.83
	2	4.83	4.83	4.83	4.83	4.83
	3	2.42	00	4.83	4.83	4.83
	4	2.42	4.83	2.42	4.83	9.66
	5	9.66	9.66	4.83	14.49	14.49
	6	4.83	4.83	4.83	9.66	4.83
	7	4.83	4.83	4.83	4.83	4.83
mean ± s.d.		4.83±2.41	4.83±2.79	4.49±0.91	7.59±3.80	6.90±3.80
B	1	4.83	9.66	19.32	24.15	4.83
	2	4.83	14.49	33.81	48.30	9.66
	3	9.66	14.49	28.98	38.64	14.49
	4	4.83	14.49	38.64	38.64	14.49
	5	4.83	9.66	24.15	48.30	14.49
	6	4.83	9.66	38.64	38.64	9.66
mean ± s.d.		5.64±1.97	12.08±2.65	30.59±7.89	39.45±8.86	11.27±3.94

Appendix Table 6.1

Peripheral eosinophil counts (cells per mm^3) of rats injected with immune rat serum (Group A), challenge controls (Group B) and rats injected with normal rat serum (Group C)

Rat No.		0 w	2 w.a.i.	4 w.a.i.	6 w.a.i.	8 w.a.i.
A	1	00	588	515	510	297
	2	00	917	928	832	186
	3	00	714	636	101	640
	4	260	1075	444	340	700
	5	292	776	430	525	156
	6	00	623	801	1248	525
	7	356	1573	840	1601	744
	8	00	864	695	1554	5712
	9	00	900	854	424	264
	10	80	384	1140	1815	744
	11	94	384	882	490	225
	12	243	898	720	1964	1133
mean ± s.d.		110±137	808±321	740±211	950±648	943±1530
B*	1	00	816	1525	1419	1704
	2	186	981	229	2145	685
	3	90	208	600	678	172
	4	231	608	2832	3270	2814
	5	198	848	1464	1392	642
	6	68	737	2717	1134	972
	7	00	1062	1169	3195	1200
	8	79	405	1680	630	2044
mean ± s.d.		106±89	708±288	1527±911	1732±1040	1279±862
C	1	00	610	465	564	520
	2	122	540	910	1080	1032
	3	00	372	848	1056	380
	4	97	1736	1736	1768	267
	5	84	873	2540	3097	783
	6	80	1056	1414	1530	984
mean ± s.d.		63±51	864±491	1318±747	1515±879	661±319

* Also used as an infection control group in experiment (5.1) carried out at the same time

Appendix Table 6.2

Serum glutamic dehydrogenase assays (micromoles product per litre of serum per minute) of rats injected with immune rat serum (Group A), challenge controls (Group B) and rats injected with normal rat serum (Group C)

Rat No.		0 w.	2 w.a.i.	4 w.a.i.	6 w.a.i.	8 w.a.i.
A	1	4.83	2.42	2.42	2.42	2.42
	2	2.42	2.42	4.83	4.83	00
	3	2.42	00	2.42	4.83	2.42
	4	2.42	2.42	4.83	4.83	00
	5	00	2.42	2.42	2.42	00
	6	2.42	00	4.83	4.83	4.83
	7	4.83	9.66	14.49	19.32	9.66
	8	00	9.66	14.49	14.49	4.83
	9	00	4.83	14.49	14.49	9.66
	10	2.42	4.83	2.42	4.83	4.83
	11	2.42	2.42	2.42	2.42	2.42
	12	4.83	4.83	4.83	4.83	4.83
mean ± s.d.		2.42±1.78	3.83±3.17	6.24±5.09	7.05±5.68	3.83±3.33
B*	1	4.83	7.25	19.32	19.32	9.66
	2	00	7.25	19.32	19.32	9.66
	3	2.42	9.66	14.49	19.32	4.83
	4	2.42	9.66	43.47	14.49	9.66
	5	2.42	9.66	19.32	9.66	9.66
	6	4.83	9.66	43.47	48.30	9.66
	7	4.83	14.49	19.32	28.98	9.66
	8	2.42	7.25	14.49	14.49	4.83
mean ± s.d.		3.02±1.71	9.36±2.39	24.15±12.11	21.74±12.11	8.45±2.24
C	1	4.83	9.66	9.66	9.66	4.83
	2	2.42	4.83	36.23	48.30	19.32
	3	4.83	4.83	9.66	9.66	9.66
	4	4.83	9.66	38.64	19.32	9.66
	5	4.83	4.83	33.81	33.81	19.32
	6	4.83	9.66	19.32	24.15	19.32
mean ± s.d.		4.83±0.98	7.25±2.65	24.55±13.35	24.15±14.97	13.69±6.42

* Also used as a infection control group in experiment (5.1) carried out at the same time

Appendix Table 6.3

Peripheral eosinophil counts (cells per mm^3) of rabbits injected with immune rabbit serum (Group A), rabbits injected with normal rabbit serum (Group B) and challenge controls (Group C)

Rabbit No.		0 w	2 w.a.i.	4 w.a.i.	6 w.a.i.	8 w a.i.	10 w.a.i.
A	1	128	338	3255	886	5482	1033
	2	00	668	1724	2688	4763	1639
	3	00	1404	3158	2448	1003	357
	4	00	202	2250	1231	2859	1046
	5	210	327	2219	3740	1571	1065
mean ±		67	587	2521	2198	3135	1028
s.d.		±97	±487	±660	±1155	±1951	±454
B	1	442	328	2944	6188	1736	1784
	2	298	175	691	3191	1273	1328
	3	00	202	1558	4014	2095	785
	4	369	119	2655	3522	1237	1615
	5	99	509	3771	2519	583	2736
mean ±		241	266	2323	3886	1384	1649
s.d.		±185	±155	±1208	±1396	±571	±715
C	1	00	140	1162	2217	999	508
	2	165	547	3211	2943	1468	558
	3	187	93	778	2553	929	446
	4	291	390	654	1304	664	253
mean ±		160	292	1451	2254	1015	441
s.d.		±120	±213	±1192	±699	±334	±133

Appendix Table 6.4

Serum glutamic dehydrogenase assays (micromoles product per litre of serum per minute) of rabbits injected with immune rabbit serum (Group A), rabbits injected with normal rabbit serum (Group B) and challenge controls (Group C)

Rat No.		0 w	2 w.a.i.	4 w.a.i.	6 w.a.i.	8 w.a.i.	10 w.a.i.
A	1	2.42	14.49	35.47	19.32	4.83	2.42
	2	4.83	12.08	31.40	33.81	14.49	2.42
	3	4.83	14.49	21.74	24.15	9.66	2.42
	4	4.83	14.49	21.74	24.15	9.66	2.42
	5	4.83	9.66	21.74	19.32	9.66	3.63
mean ±		4.35	13.04	26.42	24.15	9.66	2.66
s.d.		±1.08	±2.16	±6.57	±5.92	±3.42	±0.55
B	1	4.83	9.66	24.15	24.15	9.66	2.42
	2	2.42	9.66	38.64	38.64	9.66	4.83
	3	4.83	9.66	28.98	33.81	9.66	2.42
	4	2.42	9.66	38.64	38.64	4.83	2.42
	5	2.42	7.25	19.32	19.32	9.66	2.42
mean ±		3.38	9.18	29.95	30.91	8.69	2.90
s.d.		±1.32	±1.08	±8.64	±8.77	±2.16	±1.08
C	1	2.42	14.49	24.15	33.81	9.66	2.42
	2	2.42	14.49	33.81	24.15	9.66	4.83
	3	2.42	7.25	28.98	33.81	9.66	2.42
	4	2.42	9.66	14.49	19.32	9.66	2.42
mean ±		2.42	11.47	25.36	27.77	9.66	3.02
s.d.		±00	±3.62	±8.25	±7.24	±00	±1.21

Appendix Table 6.5

Peripheral eosinophil counts (cell per mm^3) of rabbits injected with immune rat seru (Group A), rabbits injected with normal rat serum (Group B), challenge controls (Group C), rats injected with immune rat serum (Group D) and rats injected with normal rat serum (Group E)

Animal No.		0 w.	2 w.a.i.	4 w.a.i.	6 w.a.i.	8 w.a.i.
A	1	115	00	1458	882	1062
	2	81	00	440	2224	4950
	3	00	124	444	1196	581
	4	00	535	3360	4080	1648
	5	71	492	660	1341	3900
	6	85	450	475	2142	1320
	7	00	312	606	3401	1573
mean ± s.d.		50±48	273±231	1063±1073	2180±1187	2147±1623
B	1	279	354	432	3570	976
	2	142	385	1526	4077	1234
	3	00	410	896	2864	1806
	4	89	546	1344	3297	1764
mean ± s.d.		128±117	424±85	1050±489	3452±508	1445±407
C*	1	116	525	819	894	1113
	2	00	736	336	795	1155
	3	476	960	2448	2224	868
	4	00	520	714	3640	1833
	5	00	1287	1456	3480	2355
	6	00	873	1019	2904	1768
	7	00	510	468	1912	1326
	8	00	438	720	424	2256
mean ± s.d.		74±167	731±293	997±678	2034±1248	1584±551
D	1	00	00	752	763	420
	2	128	130	855	651	93
	3	00	00	783	1792	79
	4	00	00	2599	984	308
	5	00	99	1183	1218	00
	6	00	115	2227	616	384
mean ± s.d.		21±52	57±63	1399±808	1004±447	214±178
E	1	00	531	1445	1078	798
	2	00	1074	1960	1914	705
	3	88	400	1955	1356	896
	4	00	749	1111	913	666
	5	00	484	882	2142	968
mean ± s.d.		17±39	647±271	1470±487	1480±530	806±126

* Also used as an infection control in experiment (4.4) carried out at the same time

Appendix Table 6.6

Serum glutamic dehydrogenase assays (micromoles product per litre of serum per minute) of rabbits injected with immune rat serum (Group A), rabbits injected with normal rat serum (Group B), challenge controls (Group C), rats injected with immune rat serum (Group D) and rats injected with normal rat serum (Group E)

Animal No.		0 w	2 w.a.i.	4 w.a.i.	6 w.a.i.	8 w.a.i.
A	1	2.42	4.83	9.66	14.49	24.15
	2	2.42	4.83	14.49	14.49	14.49
	3	4.83	2.42	7.25	9.66	00
	4	00	2.42	19.32	9.66	4.83
	5	2.42	2.42	14.49	24.15	4.83
	6	4.83	4.83	9.66	19.32	9.66
	7	4.83	4.83	9.66	14.49	4.83
mean ± s.d.		3.11±1.82	3.80±1.29	12.08±4.18	15.18±5.16	8.97±8.10
B	1	2.42	4.83	19.32	14.49	9.66
	2	00	4.83	14.49	14.49	9.66
	3	2.42	4.83	19.32	33.81	9.66
	4	2.42	9.66	19.32	33.81	9.66
mean ± s.d.		1.82±1.21	6.04±2.41	18.11±2.41	24.15±11.15	9.66±00
C	1	2.42	9.66	19.32	33.81	19.32
	2	2.42	9.66	19.32	33.81	9.66
	3	2.42	7.25	28.98	33.81	16.91
	4	00	4.83	19.32	24.15	2.42
	5	2.42	7.25	19.32	48.30	9.66
	6	2.42	14.49	14.49	19.32	19.32
	7	4.83	9.66	14.49	24.15	7.25
	8	4.83	14.49	30.64	63.29	19.32
mean ± s.d.		2.72±1.55	9.66±3.41	20.74±6.00	35.08±14.46	12.98±6.57
D	1	4.83	4.83	9.66	9.66	2.42
	2	4.83	19.32	9.66	24.15	00
	3	2.42	2.42	9.66	9.66	00
	4	00	4.83	9.66	19.32	2.42
	5	2.42	4.83	9.66	9.66	00
	6	2.42	4.83	9.66	9.66	2.42
mean ± s.d.		2.82±1.82	6.84±6.19	9.66±00	13.69±6.42	1.21±1.33
E	1	2.42	9.66	24.15	19.32	4.83
	2	2.42	9.66	28.98	14.49	00
	3	00	9.66	24.15	19.32	2.42
	4	4.83	9.66	28.98	24.15	2.42
	5	00	9.66	19.32	24.15	2.42
mean ± s.d.		1.93±2.02	9.66±00	25.12±4.04	20.29±4.04	2.42±1.71

Appendix Table 6.7

Peripheral eosinophil counts (cells per mm^3) of rats injected with immune rabbit serum (Group A) and rats injected with normal rabbit serum (Group B)

Rat No.		0 w	2 w.a..i.	4 w.a.i.	6 w.a.i.	8 w.a.i.
A	1	00	856	3007	3313	2351
	2	113	498	2115	1925	1233
	3	191	825	2849	2371	1766
	4	98	123	329	742	632
	5	109	121	143	339	320
mean ± s.d.		102±68	485±359	1689±1370	1738±1211	1260±825
B	1	410	727	2338	1420	2349
	2	224	891	2153	3427	3338
	3	80	430	1015	1205	890
	4	00	830	1325	1644	719
	5	00	524	737	927	449
mean ± s.d.		143±175	680±198	1514±703	1725±988	1551±1241

Appendix Table 6.8

Serum glutamic dehydrogenase assays (micromoles product per litre of serum per minute) of rats injected with immune rabbit serum (Group A) and rats injected with normal rabbit serum (Group B)

Rat No.		0 w.	2 w.a.i.	4 w.a.i.	6 w.a.i	8 w.a.i.
A	1	4.83	9.66	9.66	9.66	9.66
	2	4.83	9.66	14.49	19.32	9.66
	3	4.83	9.66	14.49	14.49	4.83
	4	4.83	4.83	4.83	4.83	4.83
	5	4.83	4.83	4.83	4.83	4.83
mean ± s.d.		4.83±00	7.73±2.65	9.66±4.83	10.63±6.30	6.76±2.65
B	1	4.83	9.66	38.64	38.64	19.32
	2	4.83	9.66	24.15	33.81	9.66
	3	9.66	19.32	38.64	48.30	19.32
	4	4.83	9.66	24.15	24.15	9.66
	5	4.83	9.66	33.81	33.81	19.32
mean ± s.d.		5.80±2.16	11.59±4.32	31.88±7.33	35.74±8.77	15.46±5.29

Appendix Table 6.9

Serum glutamic dehydrogenase assays (micromoles product per litre of serum per minute) of rats injected with immune bovine serum (Group A) and rats injected with normal bovine serum (Group B)

Rat No.		0 w.	2 w.a.i.	4 w.a.i.	6 w.a.i.	8 w.a.i.
A	1	4.83	9.66	14.49	14.49	4.83
	2	00	00	4.83	4.83	4.83
	3	4.83	9.66	38.64	14.49	4.83
	4	4.83	9.66	14.49	14.49	4.83
	5	4.83	9.66	33.81	14.49	9.66
	6	9.66	9.66	33.81	24.15	4.83
	7	9.66	9.66	28.98	28.98	4.83
	8	4.83	9.66	33.81	33.81	9.66
	9	4.83	9.66	9.66	4.83	4.83
	10	4.83	4.83	24.15	24.15	4.83
mean ± s.d.		5.31±2.74	8.21±3.26	23.67±11.93	17.87±9.67	5.80±2.04
B	1	4.83	4.83	28.98	28.98	9.66
	2	4.83	9.66	24.15	28.98	9.66
	3	9.66	9.66	24.15	43.17	9.66
	4	4.83	9.66	38.64	48.30	9.66
	5	4.83	9.66	43.47	38.64	9.66
	6	4.83	9.66	19.32	19.32	4.83
	7	4.83	9.66	19.32	19.32	4.83
	8	4.83	9.66	38.64	19.32	9.66
mean ± s.d.		5.43±1.71	9.06±1.71	29.58±9.46	30.79±11.53	8.45±2.24

Appendix Table 6.10

Serum glutamic dehydrogenase assasy (micromoles product per litre of serum per minute) of rats injected with immune ovine serum (Group A) and normal ovine serum (Group B)

Rat Group		0 w.	2 w.a.i.	4 w.a.i.	6 w.a.i.	8 w.a.i.
A	1	9.66	9.66	19.32	67.72	9.66
	2	4.83	9.66	38.64	48.30	4.83
	3	4.83	9.66	38.64	28.98	4.83
	4	00	9.66	28.98	57.96	9.66
	5	4.83	9.66	38.64	28.98	19.32
mean ± s.d.		4.83±3.42	9.66±00	32.84±8.64	46.37±17.28	9.66±5.92
B	1	9.66	14.49	38.64	24.50	9.66
	2	9.66	9.66	19.32	28.98	9.66
	3	4.83	9.66	33.81	19.32	4.83
	4	4.83	9.66	38.64	38.64	4.83
	5	4.83	9.66	48.30	38.64	9.66
mean ± s.d.		6.76±2.65	10.63±2.16	35.74±10.58	30.02±8.58	7.73±2.65

Appendix Table 6.11

Serum glutamic dehydrogenase assays (micromoles product per litre of serum per minute) of rats injected with immune ovine serum (Group A) and normal ovine serum (Group B)

Rat group		0 w	2 w.a.i.	4 w.a.i.	6 w.a.i.	8 w.a.i.
A	1	4.83	19.32	48.30	48.30	9.66
	2	9.66	9.66	28.90	48.30	9.66
	3	9.66	9.66	38.64	28.98	4.83
	4	9.66	9.66	28.98	28.98	4.83
	5	4.83	9.66	38.64	38.64	9.66
	6	9.66	9.66	38.64	38.64	9.66
	7	4.83	9.66	38.64	38.64	9.66
mean ± s.d.		7.59±2.58	11.04±3.65	37.26±6.67	38.64±7.89	8.28±2.36
B	1	9.66	19.32	38.64	48.30	19.32
	2	9.66	19.32	38.64	28.98	9.66
	3	4.83	9.66	48.30	28.98	9.66
	4	4.83	9.66	48.30	28.98	9.66
	5	4.83	9.66	28.98	48.30	9.66
	6	9.66	9.66	48.30	38.64	19.32
	7	4.83	4.83	28.98	28.98	9.66
mean ± s.d.		6.90±2.58	11.73±5.48	40.02±8.69	35.88±9.19	12.42±4.71

Appendix Table 6.12

Serum glutamic dehydrogenase assays (micromoles product per litre of serum per minute) of rats injected with γ-globulin from immune rat serum (Group A), rats injected with γ-globulin from normal rat serum (Group B),rats injected with immune rat serum (Group C) and rats injected with normal rat serum (Group D)

Rat No.		0 w	2 w.a.i.	4 w.a.i.	6 w.a.i.	8 w.a.i.
A	1	4.83	9.66	28.98	14.49	4.83
	2	4.83	9.66	14.49	14.49	9.66
	3	4.83	9.66	14.49	28.98	14.49
	4	4.83	4.83	4.83	4.83	4.83
	5	4.83	9.66	28.98	28.98	9.66
	6	00	9.66	38.64	28.98	9.66
	7	00	9.66	14.49	9.66	4.83
	8	00	4.83	4.83	4.83	4.83
mean ± s.d.		3.02±2.5	8.45±2.24	18.72±12.23	16.91±10.64	7.85±3.59
B	1	4.83	9.66	28.98	19.32	4.83
	2	4.83	14.49	48.30	38.64	19.32
	3	00	9.66	33.81	28.98	9.66
	4	4.83	9.66	48.30	48.30	19.32
	5	9.66	9.66	19.32	28.98	9.66
mean ± s.d.		4.83±3.42	10.63±2.16	35.74±12.60	32.84±11.01	12.56±6.48
C	1	00	9.66	28.98	–	9.66
	2	9.66	9.66	14.49	14.49	4.83
	3	4.83	4.83	4.83	4.83	4.83
	4	4.83	4.83	9.66	9.66	4.83
	5	4.83	4.83	19.32	19.32	4.83
mean ± s.d.		4.83±3.42	6.76±2.65	15.46±9.29	12.08±6.24	5.80±2.16
D	1	00	9.66	28.98	48.30	14.49
	2	4.83	9.66	28.98	33.81	14.49
	3	4.83	14.49	24.15	48.30	14.49
	4	9.66	14.49	48.30	19.32	9.66
	5	9.66	9.66	33.81	19.32	9.66
mean ±s.d.		5.80±4.04	11.59±2.65	32.84±9.29	33.81±14.49	12.56±2.65

Appendix Table 6.13

Serum glutmatic dehydrogenase assays (micromoles product per litre of serum per minute) of rats injected with γ-globulin from immune bovine serum and γ-globulin from normal bovine serum

Rat No.		0 w.	2 w a.i.	4 w.a.i.	6 w.a.i.	8 w.a.i.
A	1	4.83	9.66	19.32	19.32	9.66
	2	9.66	19.32	38.64	28.98	9.66
	3	9.66	9.66	28.98	19.32	9.66
	4	9.66	9.66	9.66	9.66	9.66
	5	9.66	9.66	9.66	9.66	9.66
	6	4.83	4.83	19.32	19.32	9.66
mean ± s.d.		8.05±2.49	10.47±4.75	20.93±11.29	17.71±7.27	9.66±00
B	1	4.83	9.66	24.15	24.15	9.66
	2	9.66	9.66	33.81	38.64	19.32
	3	4.83	9.66	38.64	19.32	19.32
	4	4.83	9.66	24.15	38.64	4.83
	5	9.66	19.32	48.30	41.87	9.66
	6	4.83	19.32	48.30	38.64	9.66
mean ± s.d.		6.44±2.49	12.88±4.99	36.23±10.91	33.54±9.36	12.08±5.92

Appendix Table 6.14

Serum glutamic dehydrogenase assasy (micromoles product per litre of serum per minute) of rats injected with serum from rats implanted with living flukes in chambers (Group A) and rats injected with serum from rats implanted with empty chambers

Rat No.		0 w.	2 w.a.i.	4 w.a.i.	6 w.a.i.	8 w.a.i.
A	1	4.83	4.83	4.83	4.83	4.83
	2	4.83	9.66	9.66	9.66	4.83
	3	4.83	4.83	9.66	9.66	4.83
	4	4.83	9.66	28.98	24.15	24.15
	5	9.66	9.66	9.66	4.83	4.83
	6	4.83	14.49	33.81	24.15	4.83
	7	4.83	9.66	33.81	38.64	4.83
mean ± s.d.		5.52±1.83	8.97±3.33	18.63±12.91	16.56±12.74	7.59±7.30
B	1	4.83	9.66	19.32	19.32	9.66
	2	4.83	14.49	43.47	48.30	9.66
	3	4.83	14.49	43.47	33.81	9.66
	4	9.66	9.66	48.30	14.49	14.49
	5	4.83	9.66	19.32	19.32	4.83
	6	4.83	14.49	48.30	48.30	4.83
	7	9.66	14.49	28.98	38.64	9.66
mean ± s.d.		6.21±2.36	12.42±2.58	35.88±13.04	31.74±14.18	8.97±3.33

Appendix Table 6.15

Serum glutamic dehydrogenase assays of rats injected with directly absorbed immune bovine serum (Group A), rats injected with indirectly absorbent immune bovine serum (Group B) and rats injected with normal bovine serum (Group C)

Rat No.		0 w.a.i.	2 w.a.i.	4 w.a.i.	6 w.a.i.	8 w.a.i.
A	1	4.83	28.98	33.31	38.64	19.32
	2	4.83	14.49	38.64	19.32	9.66
	3	4.83	9.66	38.64	33.81	24.15
	4	9.66	14.49	57.96	28.98	24.15
	5	9.66	28.98	38.64	38.64	14.49
	6	4.83	9.66	38.64	38.64	19.32
	7	4.83	9.66	28.98	14.49	14.49
	8	4.83	9.66	33.81	28.98	14.49
mean ± s.d.		6.04±2.24	15.70±8.45	38.64±8.55	30.19±9.20	17.51±5.11
B	1	4.83	14.49	24.15	38.64	24.15
	2	4.83	14.49	38.64	38.64	14.49
	3	4.83	14.49	33.81	33.81	9.66
	4	2.42	9.66	33.81	28.98	4.83
	5	4.83	19.32	38.64	28.98	14.49
	6	9.66	24.15	38.64	28.98	24.15
	7	4.83	14.49	38.64	33.81	14.49
	8	4.83	9.66	28.92	24.15	19.32
mean ± s.d.		5.13±2.02	15.09±4.79	34.41±5.41	32.00±5.11	15.70±6.69
C	1	4.83	9.66	48.30	38.64	9.66
	2	2.42	4.83	24.15	19.32	9.66
	3	4.83	9.66	33.81	48.30	9.66
	4	4.83	14.49	38.64	43.47	14.49
	5	4.83	14.49	43.47	48.30	9.66
	6	4.83	14.49	28.98	28.98	9.66
	7	4.83	14.49	28.98	19.32	9.66
mean ± s.d.		4.49±0.88	11.73±3.81	35.19±8.70	35.19±12.69	10.35±1.83